



Maslov, L. N., Khaliulin, I., Oeltgen, P. R., Naryzhnaya, N. V., Pei, J. M., Brown, S. A., Lishmanov, Y. B., & Downey, J. M. (2016). Prospects for Creation of Cardioprotective and Antiarrhythmic Drugs Based on Opioid Receptor Agonists. *Medicinal Research Reviews*, 36(5), 871–923. <https://doi.org/10.1002/med.21395>

Peer reviewed version

Link to published version (if available):  
[10.1002/med.21395](https://doi.org/10.1002/med.21395)

[Link to publication record in Explore Bristol Research](#)  
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Wiley at [10.1002/med.21395](https://doi.org/10.1002/med.21395). Please refer to any applicable terms of use of the publisher.

## University of Bristol - Explore Bristol Research

### General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:  
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Med. Res. Rev.

March 31, 2016 Review

**Prospects for Creation of Cardioprotective and Antiarrhythmic Drugs Based on Opioid Receptor Agonists**

Leonid N. Maslov MD, PhD, DSc<sup>1</sup>, Igor Khaliulin PhD<sup>2</sup>, Peter R. Oeltgen PhD<sup>3</sup>,  
Natalia V. Naryzhnaya MD, PhD<sup>1</sup>, Jian-Ming Pei MD, PhD<sup>4</sup>, Stephen A. Brown PhD<sup>3</sup>, Yury  
B. Lishmanov MD, PhD, DSc<sup>1,5</sup>, James M. Downey PhD, Professor of Physiology Emeritus<sup>6</sup>

<sup>1</sup>Federal State Budgetary Scientific Institution «Research Institute for Cardiology»  
Tomsk, Russia; <sup>2</sup>School of Clinical Sciences, University of Bristol, Bristol, UK; <sup>3</sup>VA Medical  
Center and University of Kentucky, Lexington, KY, USA; <sup>4</sup>Department of Physiology, Fourth  
Military Medical University, Xi'an, Shaanxi Province, China; <sup>5</sup>National Research Tomsk  
Polytechnic University, Lenina 30, 634050 Tomsk, Russia; <sup>6</sup>University of South Alabama,  
Mobile, Alabama, USA

*Correspondence to:* Igor Khaliulin, School of Clinical Sciences, University of Bristol,  
Level 7 Bristol Royal Infirmary, Upper Maudlin Street, Bristol, BS2 8HW, UK, Email:  
[i.khaliulin@bristol.ac.uk](mailto:i.khaliulin@bristol.ac.uk), Telephone: +44 117 3422208, Fax: +44 117 9299737

***Abstract***

It has now been demonstrated that the  $\mu$ ,  $\delta_1$ ,  $\delta_2$  and  $\kappa_1$  opioid receptor agonists represent the most promising group of opioids for the creation of drugs enhancing cardiac tolerance to the detrimental effects of ischemia/reperfusion. Opioids are able to prevent necrosis and apoptosis of cardiomyocytes during ischemia/reperfusion and improve cardiac contractility in the reperfusion period. The opioid receptor agonists exert an infarct-reducing effect with prophylactic administration and prevent reperfusion-induced cardiomyocyte death when ischemic injury of heart has already occurred; i.e. opioids can mimic preconditioning and postconditioning phenomena. Furthermore, opioids are also effective in preventing ischemia-induced arrhythmias.

**Key words**

Opioids, heart, ischemia/reperfusion injury, cardioprotection, signal transduction

**1. Historical Background**

The main cardiac manifestations of ischemia/reperfusion (I/R) are necrosis, apoptosis of cardiomyocytes, contractile dysfunction, and ventricular arrhythmias.<sup>1-4</sup> In the seventies it was clear that the prognosis for patients who had suffered acute myocardial infarction was highly dependent on the amount of ventricular muscle that was lost to infarction. Although it was proposed that an intervention that could reduce infarct size would save lives, there was great debate as to whether therapeutic attenuation of these negative manifestations of myocardial ischemia was even possible. This argument was settled once and for all in 1986, when three American researchers discovered the phenomenon of ischemic preconditioning (IP).<sup>5</sup> They found that exposure to 4 brief periods of I/R causes the heart to become very

resistant to infarction from a subsequent prolonged ischemic insult. The protective effect of IP is maintained only for 2 – 3 hr making it impractical for any clinical application. However, a day later these hearts again became resistant to infarction, this time lasting about 4 days. This has been variously called “delayed preconditioning”, “late preconditioning”, or “second window of protection”.<sup>6</sup> Still the major impediment to translating preconditioning to a clinical setting was the requirement that it has to be instituted prior to the onset of ischemia. Pretreatment is impossible, however, in the setting of acute myocardial infarction.

Although the mechanism of IP was obscure, it was strongly believed that it must target injury during ischemia and that the pretreatment requisite was absolute. It took another 17 years after the discovery of IP before it was realized that IP actually prevents reperfusion injury and treatment could be instituted right up to the time of reperfusion. In 2003, Vinten-Johansen’s group discovered “ischemic postconditioning” (IPost). It turned out that 3 cycles of very brief reperfusion/ischemia cycles after a prolonged ischemic insult greatly decrease the fraction of the ischemic myocardium that infarcted, often called the “infarct size-area at risk ratio” (IS/AAR).<sup>7</sup> This seminal discovery finally opened the door to clinical application but while IPost was theoretically possible in patients whose coronary thrombus was removed with angioplasty, it proved to be surprisingly awkward in many cases. A postconditioning drug would be a great improvement.

The mechanism of IP had been obscure until it was discovered that it resulted from protective signal transduction pathways triggered by Gi-coupled plasma membrane receptors.<sup>8</sup> These investigators found that adenosine was a trigger through adenosine A1 receptors but soon it was found that other Gi-coupled receptors also participated in triggering IP’s protection. In 1995, Gross’s group obtained data that the infarct-reducing effect of IP was lost after blocking opioid receptors (ORs) with naloxone<sup>9</sup> and a year later they reported that they could precondition the heart with morphine.<sup>10</sup> Kin *et al.*<sup>11</sup> demonstrated that ORs were also

involved in IPost when they showed that naloxone 5 min before reperfusion abolishes the infarct-sparing effect of IPost.

IP or IPost with ischemia is impractical in the clinical setting. The foregoing studies generated enormous interest by physiologists and pharmacologists and provided the impetus for research aimed at finding pharmaceutical OR agonists which could mimic the phenomenon of IP and IPost. In this review, we evaluate the effect of various OR ligands on the necrosis and apoptosis of cardiomyocytes, myocardial stunning, and the incidence of ischemic and reperfusion arrhythmias. To aid the reader we have included Table 1 which lists all of the OR agonists and antagonists discussed in this review.

## **2. Localization of opioid receptors involved in regulation of heart function**

### ***A. Opioid receptors in the central nervous system***

It is well known that all discovered mammalian opioid peptides have been isolated from brain where they are most abundant<sup>12</sup> and it is not surprising that the brain and spinal cord have a high density of ORs.<sup>13-21</sup>  $\mu$  OR were discovered in the spinal cord, in the periaqueductal grey matter (PAG), nucleus accumbens, amygdala and in several thalamic nuclei.<sup>15, 19</sup> Transcripts of  $\mu$  OR were found in the prefrontal cortex, nucleus accumbens, caudate putamen and thalamus.<sup>20</sup>  $\delta$  OR was identified in spinal cord,<sup>16</sup> caudate putamen, nucleus accumbens and olfactory tubercle.<sup>18</sup> Transcripts of  $\delta$  OR were detected in the prefrontal cortex, nucleus accumbens, caudate putamen.<sup>20</sup> The  $\kappa$  OR was found in spinal cord.<sup>16</sup> Transcripts for this receptor were identified in nucleus accumbens, caudate putamen, preoptic area and hypothalamus.<sup>20</sup>  $\kappa$  OR were also found in the prefrontal cortex, nucleus accumbens, hypothalamus, amygdala, ventral tegmental area, dorsal raphe nucleus, and locus coeruleus.<sup>21</sup> The ORL1 receptor or nociceptin/orphanin FQ (N/OFQ) opioid peptide receptor (NOPr) was found in several rat brain areas, including the cerebral cortex, thalamus, subfornical organ, habenula, hypothalamus, central gray, dorsal raphe, locus coeruleus hippocampus,

amygdala, caudate nucleus, putamen, medial thalamic nuclei and the dorsal horn of the spinal cord.<sup>14,17</sup>

Most of opioid peptides do not penetrate the blood-brain barrier so their effects, when administered intravenously, are associated with activation of peripheral ORs.<sup>22-24</sup> However, the non-peptide OR agonists can enter the brain and activate ORs in autonomic centers regulating the functional state of the heart. It has been shown that perfusion of the fourth cerebral ventricle with the selective peptide  $\mu$  OR agonist FK 33-824 induces bradycardia in the conscious dogs.<sup>25</sup> In anesthetized dogs, [D-Met<sup>2</sup>,Pro<sup>5</sup>]enkephalinamide perfusion through the cerebroventricular system elicited bradycardia, which was accompanied by an increase in the vagal discharge rate.<sup>26</sup> It has been shown that intracisternal administration of opioid peptides also evoked bradycardia in unanaesthetized dogs.<sup>27</sup> This effect was abolished by pretreatment with atropine. It has also been found that intracerebroventricular administration of the selective  $\mu$  OR agonist DAMGO or the selective  $\delta$  OR agonist DPDPE increased plasma catecholamine levels and blood pressure in conscious rats.<sup>28</sup> However DAMGO appeared to be a more potent regulator of the catecholamine level than DPDPE. At a dose of 5 nM and higher, DAMGO induced bradycardia mediated by vagal activation. The authors concluded that brain ORs regulating autonomic outflow, cardiovascular and respiratory function are mainly of the  $\mu$  type, although a  $\delta$  opioid system may also contribute to sympathoadrenal and respiratory effects of opioids.

Thus, presented data indicate that ORs are present in the brain regions responsible for the regulation of function of the cardiovascular system and the stress response to strong stimuli.

### ***B. Opioid receptors in the heart***

All three OR ( $\mu$ ,  $\delta$ ,  $\kappa$ ) transcripts were also detected in several peripheral tissues including the intestine, adrenal, kidney and lung.<sup>29</sup> In the stomach,  $\delta$  OR and  $\kappa$  OR but not  $\mu$

OR transcripts were found.<sup>29</sup> mRNAs for opioid precursors were detected in adrenocortical cells.<sup>30</sup> It has been established that  $\mu$  and  $\kappa$  OR agonists can regulate cortisol and aldosterone secretion from the adrenocortical cells.<sup>30</sup> The  $\delta$  OR was found in a PC12 cell line derived from a pheochromocytoma of the rat adrenal medulla.<sup>31</sup> Changes in function of these organs by activation of their ORs may indirectly affect the heart's function.

The first report reporting the existence of ORs in the myocardium was published in 1981.<sup>32</sup> In 1988, the existence of  $\delta$  OR in the myocardium was demonstrated using a radioligand binding assay.<sup>33</sup> The next year,  $\delta$  and  $\kappa$  ORs were also found in rat cardiac sarcolemma using this method.<sup>34</sup> Later other investigators<sup>35</sup> confirmed the existence of  $\kappa_1$  OR in the myocardium.<sup>36</sup> Opioid-binding sites in the myocardium were also confirmed in other studies.<sup>37,38</sup> In 1996, transcripts of  $\delta$  and  $\kappa$  ORs were found in the heart.<sup>29</sup> These data were later confirmed by Weil *et al.*<sup>39</sup> None of these studies detected the  $\mu$  OR in cardiomyocytes. However, in 1995, the  $\mu_3$  subtype of this receptor () was detected in the coronary microvascular's endothelial cells.<sup>40</sup> This group of researchers also established that endothelial cells express a  $\delta_2$  OR subtype.<sup>41</sup> Vascular smooth muscle cells also appeared to express  $\delta$  OR.<sup>42</sup> Dumont and Lemaire were able to detect and characterize a high affinity [<sup>3</sup>H]nociceptin binding site in the membrane preparations of rat heart.<sup>43</sup> Kim *et al.* confirmed the existence of the ORL1 receptor in cardiac myocytes.<sup>44</sup> Thus, the view was formed that cardiac myocytes express  $\delta$  OR,  $\kappa$  OR and ORL1 receptor but not  $\mu$  OR. This opinion was changed in 2005 when Head *et al.* found  $\mu$  OR on the sarcolemma of cardiomyocytes using immunofluorescence microscopy.<sup>45</sup>  $\mu$ -OR mRNA was also identified in the human right atrium. However, the amount of this receptor's mRNA in cardiomyocytes was significantly lower than the ORL1 mRNA content.<sup>46</sup> Later,  $\mu$ ,  $\delta$  and  $\kappa$  ORs were detected immunohistochemically in human heart.<sup>47</sup> The researchers found that  $\mu$  and  $\delta$  ORs are located mainly in cardiomyocytes as well as on sparse individual nerve fibers. Likewise,  $\kappa$  OR was

identified predominantly in cardiomyocytes. This receptor was also found on intrinsic cardiac adrenergic cells. It has been established that the  $\delta$  OR is co-localized with the sensory neuron marker calcitonin gene-related peptide (CGRP).<sup>47</sup> Previously, similar data were obtained by Mousa *et al.* They identified  $\mu$  OR and  $\kappa$  OR mRNA, as well as other OR proteins on cardiac parasympathetic, sympathetic, and sensory neurons.<sup>48</sup>  $\delta$  ORs were detected in the cholinergic neurons, small intensely fluorescent (SIF) catecholaminergic cells, afferent nerve terminals, and atrial cardiomyocytes.<sup>49</sup>

Thus, all four types of ORs ( $\mu$ ,  $\delta$ ,  $\kappa$  and ORL1) have been found in cardiomyocytes.  $\delta$  OR,  $\kappa$  OR and ORL1 receptor appeared to have the highest density in cardiomyocytes.  $\mu$  and  $\delta$  ORs are present in the endothelial cells and vascular smooth muscle cells express  $\delta$  OR. ORs have been detected on the sensory nerve terminal, on intrinsic cardiac adrenergic cells and are probably present in the sympathetic and parasympathetic terminals in the heart. It is safe to assume that activation of any of these receptors may potentially affect the functional state of the heart.

Intrinsic cardiac adrenergic (ICA) cells were identified in rodent and human heart by Huang *et al.*<sup>50</sup> In 2007, they discovered localization of  $\delta$  OR immunoreactivity in ICA cells in human and rat hearts.<sup>51</sup> They demonstrated that the selective  $\delta_1$  OR agonist DPDPE enhanced epinephrine<sup>51</sup> and CGRP release<sup>52</sup> from ICA cells in denervated rat heart and these effects were abolished by the  $\beta$ -adrenergic and CGRP receptor inhibitors. The authors suggested that the cardiotropic effects of  $\delta$  OR agonists are mediated through  $\beta_2$ -AR/CGRP signaling.

### ***C. Opioid receptors modulate neural control of the heart***

Opioid peptides can alter the autonomic nervous regulation of the heart function. Indeed, Kett *et al.* established that intravenous administration of H-Tyr-D-Arg-Phe-Lys-NH<sub>2</sub> (DALDA), a selective peptide  $\mu$  OR agonist which does not penetrate the blood-brain barrier, blunted norepinephrine-induced baroreflex bradycardia but had no effect on the sodium



nitroprusside-evoked tachycardia.<sup>53</sup> Pretreatment with naloxone methiodide, a peripheral OR antagonist, abolished DALDA-induced suppression of baroreflex. These data indicate that DALDA inhibits the baroreflex through peripheral OR occupancy. Later, these investigators established that the selective peptide  $\mu$  OR agonist D-Ala<sup>2</sup>,N-Me-Phe<sup>4</sup>,Gly<sup>5</sup>-ol (DAMGO) suppresses baroreflex-mediated bradycardia in the awake sheep but the selective  $\kappa$  OR agonist U50,488 had no such effect.<sup>54</sup> Peripheral  $\mu$  OR stimulation can suppress vagus-mediated baroreflex and it can be assumed that these ORs are located in the nerve endings innervating the sinoatrial node.

Urthaler *et al.* established that selective perfusion of the sinus node with morphine in anesthetized dogs evokes bradycardia.<sup>55</sup> Bradycardia was not altered by atropine or vagotomy and intranodal administration of morphine had no effect on the acceleration of heart rhythm produced by stellate ganglion stimulation or by selective perfusion of the sinus node with norepinephrine. The authors concluded that morphine-evoked bradycardia was autonomic nervous system independent and a direct effect of morphine on the sinoatrial node cells. These results were confirmed by the data of Gautret and Schmitt.<sup>56</sup> They found that an intravenous administration of ethylketocyclazocine, a preferential  $\kappa$  OR agonist, induced a fall in heart rate and blood pressure (BP) in rats anaesthetized with pentobarbital. The bradycardia and the hypotension were not altered by bilateral vagotomy and atropine, but were completely eliminated by naloxone and Mr 2266, a preferential  $\kappa$  OR antagonist. Ethylketocyclazocine-induced bradycardia persisted in  $\beta$ -adrenoreceptor-blocked and pithed rats.<sup>56</sup> These results indicate that peripheral  $\kappa$  OR located in the heart's conduction system can affect cardiac rhythm.

However, other data indicate that opioids can exhibit vagolytic effect. The *nervi vagi* of isolated perfused rabbit heart were electrically stimulated and morphine, a preferential  $\mu$  OR agonist, met-enkephalin,  $\mu$  OR and  $\delta$  OR agonist, and D-Ala<sup>2</sup>,D-Leu<sup>5</sup>-enkephalin

(DADLE), a preferential  $\delta$  OR agonist, reduced the vagal bradycardia with  $IC_{50}$  values of 148, 25 and 3.2 nM, respectively. Pretreatment with naloxone abolished the vagolytic effect of all opioids. The selective  $\delta$  OR antagonist ICI 174864 eliminated met-enkephalin effect but did not antagonize morphine's action.<sup>57</sup> These data indicate that stimulation of both  $\mu$  OR and  $\delta$  OR can attenuate vagus-mediated bradycardia but stimulation of presynaptic  $\delta$  OR have a more powerful vagolytic effect. Similar data were obtained by Musha *et al.* in the experiments on anesthetized dogs with electrical stimulation of *n. vagus*.<sup>58</sup> They confirmed that presynaptic  $\delta$  OR activation prevents vagal bradycardia. In pithed rats pretreated with propranolol, vagal stimulation or injection of methacholine decreased heart rate (HR).<sup>59</sup> The selective ORL1 receptor agonist nociceptin (orphanin FQ) decreased the vagal bradycardia but did not modify the methacholine-induced decrease in HR. The selective ORL1 receptor antagonist [Phe<sup>1</sup> $\psi$ (CH<sub>2</sub>-NH)Gly<sup>2</sup>]-nociceptin(1-13)NH<sub>2</sub> antagonized vagolytic effect of nociceptin. Authors concluded that orphanin FQ prevents vagal bradycardia acting on the presynaptic ORL1 receptor located on the vagal terminal in the heart.<sup>59</sup> It was established that MEAP (met-enkephalin-Arg-Phe) and the selective  $\delta_2$  OR agonist deltorphin II suppressed vagal bradycardia when they were delivered directly into the sinoatrial node by local microdialysis.<sup>60</sup> The authors also found that  $\delta$  OR stimulation only in the sinoatrial node prevents vagal bradycardia. In the further study, they conducted a comparative analysis of the ability of  $\delta$  OR agonists to suppress vagal bradycardia during administration into the sinoatrial node and found that the vagolytic effect of opioids is mediated by  $\delta_2$  ORs in the sinoatrial node.<sup>61</sup> These data were confirmed in a subsequent study by the same group.<sup>62</sup> They later established that  $\delta_2$  ORs are located on the cholinergic vagal terminals in the sinoatrial node.<sup>63</sup>

Opioids can modulate not only the vagal discharge rate but also sympathetic outflow. Ledda and Mantelli using isolated guinea-pig atria discovered that the non-selective OR agonist etorphine inhibits the sympathetic response induced by direct electrical stimulation.<sup>64</sup>

Pretreatment with naloxone abolished this effect of etorphine. However, etorphine did not affect an inotropic effect of norepinephrine. Authors concluded that etorphine stimulates presynaptic inhibitory ORs on adrenergic nerve terminals in the heart. Later they established that the inhibitory effect of the opioid peptides could be due to stimulation of presynaptic inhibitory  $\delta$  and  $\kappa$  ORs on adrenergic nerve terminals in the heart.<sup>65, 66</sup> Somewhat different results were obtained by Starke *et al.*<sup>67</sup> In their study using selective OR agonists and antagonists they found that, under *in vitro* conditions, only presynaptic  $\kappa$  ORs but not  $\mu$  ORs or  $\delta$  ORs inhibit the norepinephrine release from the sympathetic nervous innervating the rabbit heart. Fuder published results of experiments where isolated guinea-pig atria were loaded with  $^3\text{H}$ -(-)-norepinephrine. In these experiments, the intrinsic nerves stimulation evoked norepinephrine efflux.<sup>68</sup> They discovered that the non-selective OR agonist etorphine, the  $\kappa$  OR agonists ethylketocyclazocine, dynorphin A (1-13) and the  $\delta$  OR agonist DADLE but not the preferential  $\mu$  OR agonist morphine inhibit the stimulation-induced norepinephrine efflux in a concentration-dependent manner. The inhibitory effect of ethylketocyclazocine and etorphine was antagonized by naloxone. The authors hypothesized that activation of presynaptic  $\kappa$  ORs and apparently  $\delta$  ORs inhibits norepinephrine release from sympathetic nerves in the heart.<sup>68</sup> Others showed that a strong inhibition of on the sympathetic-mediated positive inotropic effect evoked by electrical field stimulation of guinea-pig atria can be achieved by  $\kappa$  OR agonists U-50488 and U-69593, whereas  $\delta$  OR agonists, DPDPE and BW373U86 were ineffective. This effect of  $\kappa$  OR agonists was reversed by the selective  $\kappa$  OR antagonist nor-binaltorphimine.<sup>69</sup>

Similar data were obtained in the *in vivo* experiments on pithed animals. Thus in pithed rabbits, it was found that ethylketocyclazocine decreased blood pressure (BP), the endogenous plasma norepinephrine level and the  $^3\text{H}$ -norepinephrine release rate.<sup>70</sup> These effects were inhibited by naloxone. Investigators concluded that ethylketocyclazocine inhibits

norepinephrine release from postganglionic sympathetic neurons, apparently by stimulation of ORs at the terminal axons. Later they established that the preferential  $\kappa_2$  OR agonist bremazocine prevents the 3H-norepinephrine release and BP elevation in response to electrically (2 Hz) stimulated sympathetic outflow in pithed rabbits but has no effect on the BP increase evoked by an intravenous infusion of norepinephrine.<sup>71</sup> The inhibitory effects of bremazocine was antagonized by naloxone. These results indicate that the  $\kappa_2$  OR stimulation inhibits norepinephrine release and consequently lowers BP by activation of peripheral, probably prejunctional,  $\kappa$  OR. This function of  $\kappa$  OR was later confirmed in the experiments of Caffrey's group<sup>72</sup> whilst Feuerstein *et al.* excluded a possible role of  $\mu$  OR in the regulation of sympathetic outflow in pithed rats.<sup>73</sup> Malinowska *et al.* showed in the experiments on pithed rats pretreated with atropine that the postganglionic sympathetic nerves innervating the rat heart have presynaptic ORL1 receptor and its activation inhibits the sympathetic outflow.<sup>59</sup> Thus, the data show that activation of peripheral ORs can inhibit the cardiotropic effects of parasympathetic and sympathetic nerve stimulation. However, in the conscious animals the effect of opioid peptides can be quite the opposite. In particular, it has been shown that in unanesthetized sheep and dogs, intravenous administration of enkephalins or nociceptin may cause transient rise in BP and HR associated with enhanced sympathetic outflow.<sup>74,75,76</sup> These effects were associated with activation of ORs located outside the blood-brain barrier in the area postrema, a blood-brain barrier-deficient small, elevated area in the lateral wall of the inferior recess of the fourth ventricle.<sup>74-76</sup> It was established that stimulation of central  $\mu$  and  $\delta$  OR also can increase plasma catecholamine levels and BP.<sup>12,28</sup>

In 1990, Giuliani *et al.* demonstrated that electrical stimulation of the left atria of reserpine-pretreated guinea-pigs in the presence of atropine produces a positive inotropic effect involving activation of capsaicin-sensitive afferents.<sup>77</sup>  $\mu$  OR agonists dermorphin, DAMGO and morphine all inhibited this effect. The authors concluded that capsaicin-

sensitive nerves in the atrium have  $\mu$  OR, which inhibit transmitter release from sensory nerve terminals.<sup>77</sup> In a similar model, these investigators found that the selective ORL1 agonist nociceptin inhibits a positive inotropic response induced by electrical field stimulation.<sup>78</sup> However, nociceptin (the selective ORL1 agonist) did not affect the positive inotropic effect of exogenous CGRP. Therefore, the authors suggested that nociceptin inhibits CGRP release by activation of ORL1 receptors localized on the afferent nerve endings in atria.<sup>78</sup>

The adrenal medulla can also be involved in the cardiovascular effects of opioids. Gulati and Bhargava studied cardiovascular effects of intravenous administration of  $\kappa$  OR agonists bremazocine, tifluadom and U-50,488 in anesthetized rats.<sup>79</sup> All three opioids evoked bradycardia. Bremazocine and U-50,488 decreased BP. The hemodynamic effects of the opioids were blocked by bilateral adrenal demedullation. The peripherally acting OR antagonist naltrexone methylbromide blocked the cardiovascular effects of U-50,488. Based on these results, the investigators suggest that cardiovascular effects of  $\kappa$  OR agonists are mediated through the adrenal medulla and peripheral  $\kappa$  OR stimulation.<sup>79</sup> The mechanism of this effect of  $\kappa$  OR agonists remains unknown.

Taken together, the available experimental data suggest that the heart is richly populated with ORs located on the sarcolemma of cardiomyocytes, cell membrane of ICA and the coronary endothelial cells. In addition they are located on the sympathetic and parasympathetic nerve terminals in the heart, in the adrenal medulla and in the brain regions responsible for the regulation of the heart. Thus it should come as no surprise that some of these can exert a cardioprotective effect against ischemia/reperfusion injury.

### **3. Anti-Infarct Effect of Pretreatment with Opioid Receptor Agonists**

#### ***A. $\delta_1$ opioid agonists***

Rats given 0.3 mg/kg of morphine intravenously prior to coronary artery occlusion/reperfusion experienced a decrease in the IS/AAR by 4.5 fold.<sup>10</sup> A year later, the

same group of researchers found that the infarct-sparing effect of morphine depended upon  $\delta$  OR activation.<sup>80</sup> In 1998, Miki *et al.* reported that morphine reduced infarct size in rabbits.<sup>81</sup> Morphine was tested at doses of 0.3, 0.8 and 3 mg/kg but only the highest dose protected suggesting a species difference between ORs in rats and rabbits. Morphine also increases the tolerance of isolated rat cardiomyocytes to a 90-min hypoxia.<sup>82</sup> Wu *et al.*<sup>83</sup> confirmed the cardioprotective properties of morphine. They administered 8 mg/kg intraperitoneally to rats. It was not mentioned why they selected such a high dosage but they found that morphine's protection could be prevented by blocking  $\mu$ ,  $\delta$  or  $\kappa$  ORs suggesting that all three OR subtypes seem to be involved in the cardioprotection of morphine. Lu *et al* corroborated the infarct-sparing effect of morphine in rats at 0.3 mg/kg intravenously.<sup>84</sup>

Bilir *et al.* showed that tramadol, an agonist and antagonist of ORs, increases the isolated rat heart's tolerance to I/R.<sup>85</sup> In a clinical trial tramadol was given prior to coronary artery bypass surgery.<sup>86</sup> Surprisingly, tramadol caused an increase in a marker of cardiomyocyte necrosis, cardiac troponin I (cTnI) in the blood of patients suggesting that this opioid actually exacerbates injury of the heart during coronary artery bypass surgery. This demonstrates why results of any animal study must be tested in clinical trials.

Irwine's group was the first to demonstrate remifentanyl induced cardioprotection in both *in vivo* and isolated heart models.<sup>87,88</sup> The infarct-reducing effect of remifentanyl was abolished by pretreatment with the selective  $\kappa$  OR antagonist nor-binaltorphimine and the selective  $\delta$  OR antagonist naltrindole. Later the cardioprotective effect of remifentanyl was confirmed in an isolated perfused rat heart.<sup>89</sup> In 2010, a clinical trial of remifentanyl was carried out.<sup>90</sup> Forty patients with on-pump coronary artery bypass surgery were included in this trial. All patients were anesthetized with propofol and pretreated with fentanyl. Some of the patients (n = 20) received remifentanyl (1  $\mu$ g/kg intravenously and then infusion with rate of 0.5  $\mu$ g/kg during 30 min) prior to surgery. Cardioprotection was determined 24 hr

postoperatively by assessing biochemical markers of myocardial necrosis: creatine kinase MB (CK-MB) and cTnI. CK-MB and cTnI levels were significantly lower in patients that received remifentanyl.<sup>90</sup> Thus, unlike tramadol, remifentanyl appears to be cardioprotective not only in animals but also in patients with I/R injury of heart.

Pretreatment with the selective  $\delta_1$  OR agonist TAN-67 (10 mg/kg intravenously) decreased the IS/AAR in rats and the selective  $\delta_1$  OR antagonist BNTX abolished the effect.<sup>91</sup> This experiment indicated that the  $\delta_1$  OR was protective and a year later, using an isolated perfused heart, it was shown that the  $\delta$  OR-selective agonist DADLE could also protect.<sup>92</sup> More recent studies indicate that 10 mg/kg DADLE prior to coronary artery occlusion decreases IS/AAR and the highly selective  $\delta$  OR antagonist naltrindole abolished this effect.<sup>93</sup> The cardioprotective effect of DADLE was confirmed in later investigations.<sup>94, 95</sup> In vivo, this peptide exhibited an infarct-reducing effect in rats at a dose of 1 mg/kg.<sup>94</sup> It was also found that the  $\mu$  OR-selective agonist methadone (0.3 mg/kg) shows an infarct-reducing effect, which is actually mediated via  $\delta$  OR activation.<sup>96</sup>

Takasaki *et al.* found that cardiomyocytes tolerance to hypoxia/reoxygenation is increased after addition the  $\mu$  and  $\delta$  OR agonist met-enkephalin to the incubation buffer.<sup>97</sup> Later, this team of investigators using naltrindole showed that the cytoprotective effect of met-enkephalin is mediated via  $\delta$  OR occupancy.<sup>98</sup> Infusion of met-enkephalin to rabbits starting 24 hr before coronary artery occlusion with an osmotic minipump promoted a decrease in the IS/AAR by 60%.<sup>99</sup> However, a 24-hr infusion of met-enkephalin in mice failed to reduce the infarct size.<sup>100</sup> This indicates again that there are species differences in the response to some opioids. This is most likely due to small but important differences in the genetic codes for these receptors among the species. In *in vivo* experiments with pigs, researchers could not demonstrate an infarct-reducing effect of DADLE at a dose of 1 mg/kg intravenously.<sup>101</sup>

The ability of the  $\delta_1$  OR agonist TAN-67 to mimic the cardioprotective effect of preconditioning in rat heart was confirmed in the later studies both *in vivo*<sup>102</sup> and *in vitro*<sup>103-105</sup>. We established that perfusion of the isolated rat heart with the  $\delta_1$  OR-selective agonist DPDPE (154 nM) decreases reperfusion-induced creatine kinase release.<sup>106</sup> Pretreatment with the  $\delta$  OR-selective antagonist naltrindole (1 nM) completely abolished DPDPE's cardioprotective effect. In 2001, McPherson and Yao<sup>107</sup> showed that the  $\delta$ -selective agonist BW373U86 (10 pM) increases tolerance of isolated cardiomyocytes to hypoxia/reoxygenation. The cardioprotective property of TAN-67 and BW373U86 were confirmed *in vivo* at coronary artery occlusion and reperfusion.<sup>108,109</sup> In addition, it was established that the infarct-sparing effect of BW373U86 (1 mg/kg) is a consequence of  $\delta_1$  OR activation<sup>109</sup>.

In pigs, an infarct-reducing effect of DPDPE was found at a dose of 1 mg/kg intravenously<sup>101</sup> but this dose was not protective in rats.<sup>110,111</sup> Again, a species difference was present. DPDPE at the final concentration of 0.1 mg/L (154 nM) did protect the isolated perfused rat heart<sup>112,113</sup> and DPDPE's protection in rat heart can be blocked by naltrindole.<sup>104,106,114</sup> The infarct-sparing effect of DPDPE was confirmed in experiments in the isolated rat heart by M.H. Huang *et al.*<sup>52</sup> In 2006, Watson *et al.* reported that the  $\delta_1$  and  $\delta_2$  OR agonist ARD-353 (0.3 mg/kg) decreased the IS/AAR in rats.<sup>115</sup> This effect disappeared after  $\delta_1$  OR inhibition with BNTX. In addition, Watson *et al.* obtained data that ARD-353 does not penetrate through the BBB.<sup>115</sup> These authors concluded that the cardioprotective effect of ARD-353 is a consequence of peripheral  $\delta_1$  OR activation. In 2006, E.R. Gross *et al.*<sup>116</sup> reported that the  $\delta$  OR agonist fentanyl isothiocyanate caused infarct reduction at a dose of 10  $\mu$ g/kg intravenously. Hence, there is a reason to believe that the  $\delta_1$  OR agonists are excellent candidates for cardioprotective drug development.

### ***B. $\delta_2$ opioid agonists***



In 2002, in experiments with pigs, we demonstrated the infarct-reducing effect of the putative  $\delta_2$  OR agonist deltorphin D at a dose of 1 mg/kg.<sup>101</sup> In 2009, in experiments with rats, we confirmed these data.<sup>117</sup> The selective  $\delta_2$  OR agonist deltorphin II at a dose of 0.12 mg/kg can decrease the IS/AAR. The infarct-sparing effect of deltorphin II was maintained in the presence of the  $\delta_1$  OR antagonist BNTX but disappeared after  $\delta_2$  OR block with naltriben.<sup>117</sup> The cardioprotective effect of deltorphin II was abolished after blocking peripheral OR with naloxone methiodide (5 mg/kg). Hence, the  $\delta_2$  OR also seemed to be protective.

### *C. $\kappa$ opioid agonists*

The  $\kappa_1$  OR-selective agonist U50,488 increases the tolerance of isolated cardiomyocytes to sodium cyanide toxicity in the incubation buffer.<sup>118</sup> They showed that U50,488 (10  $\mu$ M) decreases the IS/AAR in the isolated perfused rat heart. We confirmed that pretreatment with U50,488 protects the isolated perfused rat heart to global I/R.<sup>113,119,120</sup> Addition of  $\kappa$  OR-selective agonist dynorphin to incubation buffer increases tolerance of rabbit cardiomyocytes to 3 h of hypoxia and the  $\kappa$  OR-selective antagonist GNTI abolished dynorphin's protection.<sup>98</sup> In 2004, it was shown that pretreatment with the  $\kappa$  OR agonists U50,488, ICI 204,448 and BRL 52537 exhibit infarct-reducing effect in vivo.<sup>121</sup> The  $\kappa$  OR-selective antagonist nor-binaltorphimine abolished the infarct-sparing effect of U50,488 and ICI 204,448 but did not affect the cardioprotective effect of BRL 52537.<sup>121</sup> Since ICI 204,448 does not penetrate through the BBB,<sup>122</sup> it is likely that the peripheral  $\kappa$  OR activation promotes the protection. The anti-necrotic effect of U50,488 is seen in the isolated heart. Recently, we have found that the quaternary ammonium salt of U50,488 (Q-U50,488) which is not able to pass the BBB, elicits a protective effect against cardiac I/R injury.<sup>123</sup> The infarct-sparing effect of Q-U50,488 was abolished by nor-binaltorphimine implicating a peripheral  $\kappa$  OR. It can safely be assumed that the  $\kappa$  OR regulating cardiac tolerance to ischemia/reperfusion is located in the heart.

#### ***D. $\mu$ opioid agonists***

Using isolated perfused rat heart we found that the  $\mu$  OR-selective agonist DAMGO reduces infarction after global I/R.<sup>124</sup> The protection from DAMGO was confirmed in our later investigations.<sup>125,126</sup> In addition, we established that the  $\mu$  OR-selective agonist DALDA also can prevent cardiac cell death during global I/R. However, intravenous DAMGO (0.1 mg/kg) or DALDA (0.1 mg/kg) 15 min prior to heart isolation actually increased the injury from I/R *ex vivo*.<sup>125-127</sup> Gross's group found that DAMGO (0.1 mg/kg intravenously) had no effect on the IS/AAR in rats after I/R *in vivo*.<sup>128</sup> In our *in vivo* investigation in rats with coronary artery occlusion (45 min) and reperfusion (2 h), we studied the  $\mu$  OR-selective agonist dermorphin H (0.12 mg/kg) and DAMGO (0.08 or 0.8 mg/kg).<sup>110,111</sup> Neither of these peptides had any effect on the IS/AAR. It remains unclear why DAMGO is so protective *ex vivo* but not *in vivo*.

Gross *et al.* found that the  $\mu$  and  $\delta$  OR-selective agonist Eribis peptide 94 starting 10 min after coronary artery occlusion (30 min) and continuing during reperfusion decreases the IS/AAR in open-chest rats. This protection persisted after inhibition of  $\delta$  OR with naltrindole,  $\delta_1$  OR with BNTX and  $\kappa$  OR with nor-binaltorphimine. However, it was abolished with the selective  $\mu$  OR antagonist CTOP.<sup>129</sup> This was the first convincing evidence that  $\mu$  OR activation protects the heart from ischemia/reperfusion.

#### ***E. ORL1 opioid agonists***

Recently, we evaluated a fourth OR subtype which usually denoted as ORL1 receptor (opioid-like receptor 1) in an *in vivo* rat model using the endogenous ORL1-selective agonist Nociceptin. Neither 0.4 or 2.2 mg/kg had any effect on the IS/AAR.<sup>110,111</sup> In our opinion, it is too early to draw a final conclusion that ORL1 receptors do not affect the heart's tolerance to I/R because we have not yet studied it in the isolated heart. The  $\mu$  OR agonists are

cardioprotective *ex vivo* but not *in vivo* and it is possible that nociceptin may act in a similar fashion.

#### **4. Toxicity of High Dose Opioids and the Cardioprotective effects of OR Antagonists**

The aforementioned studies demonstrate that OR activation can increase the heart's tolerance to ischemia/reperfusion but there are some studies demonstrating that OR stimulation can also exacerbate I/R injury. Intravenous administration of morphine at a dose of 2.1 mg/kg can induce ST segment depression in patients with ischemic heart disease.<sup>130</sup> The authors interpreted this effect as a manifestation of myocardial ischemia. In another study, it was shown that morphine at a dose of 1 mg/kg increases ST segment elevation in cats with coronary artery ligation, which they regarded as worsening of the heart's ischemia.<sup>131</sup> In 1982, the same group of authors did obtain data that morphine can increase the infarct size in rats.<sup>132</sup> Morphine was administered at a dose of 3 mg/kg intravenously for 10 min prior to a 48-h coronary artery occlusion without reperfusion. Permanent occlusion without reperfusion in rodent hearts is now considered as an invalid methodology for evaluating cardioprotection since cardiac muscle cannot survive in the complete absence of blood flow. In addition, these data contradict the results of the Chinese investigators, which showed that morphine at a dose of 8 mg/kg evokes a decrease in the IS/AAR.<sup>83</sup> We tested 0.3, 0.8. and 3.0 mg/kg morphine pretreatment in open-chest rabbits and found no effect of the two lower doses but greatly reduced infarct size with the high dose.<sup>81</sup>

In 1985, it was reported that 1.1 or 3.6 mM naloxone in the perfusion solution protects the isolated heart.<sup>133</sup> Naloxone's IC<sub>50</sub> towards  $\mu$  and  $\delta$  OR is 8.2 nmol.<sup>134</sup> Similarly, the K<sub>i</sub> of naloxone towards  $\mu$  OR is reported to be 3.4 nmol but that towards  $\delta$  OR is 50 nmol.<sup>135</sup> We would suggest that the cardioprotective effect of their very high-dose naloxone is probably a

non-specific membrane stabilizing effect of the drug, rather than a consequence of the blockade of ORs. It should also be noted that in most of our experiments we have not observed infarct-reducing effect of naloxone, naltrexone and most of other OR antagonists.<sup>81,110</sup> Similarly, many other investigators failed to observe a cardioprotective effect of the OR antagonists *in situ* or *in vitro*. An exception is the work of Chen *et al.* They performed 45 min global ischemia and 60 min reperfusion of an isolated rat heart in which OR antagonists were added to perfusion buffer for the first 10 min of reperfusion. Necrosis was evaluated by IS/AAR and by monitoring CK-MB levels in coronary effluent. Naloxone (10 nM), naltrindole (5 nM), or nor-binaltorphimine (5 nM) decreased the IS/AAR and CK-MB release.<sup>136</sup> Hence, these OR antagonists mimic IPost phenomenon. The concentrations of antagonists indicators used approach their published  $K_i$  and  $IC_{50}$ .<sup>134,135,137</sup> Therefore, we cannot easily dismiss the cardioprotective effect of OR antagonists as a nonspecific effect. We found that intravenous administration of the  $\mu$  OR antagonist CTAP (1 mg/kg) to rats prior to coronary artery occlusion (20 min) and reperfusion (3 h) promotes a decrease in the IS/AAR.<sup>138</sup> However, protection may have been mediated via the somatostatin receptor for which this peptide exhibits moderate affinity.<sup>139</sup> Somatostatin is known to limit the IS/AAR in *in vivo* studies.<sup>140</sup>

Our investigations do indicate the existence of an OR pool, or non-ORs, whose activation with opioids negatively affects cardiac tolerance to I/R.<sup>104,112</sup> In isolated rat heart studies, we observed that the cardioprotective effect of the  $\delta_1$  agonist DPDPE disappears if the concentration of peptide in the perfusion buffer is increased to 740 nM.<sup>104,112</sup> In isolated murine heart, 10  $\mu$ M of morphine was not protective<sup>141</sup> while 0.3  $\mu$ M did protect isolated rabbit heart.<sup>81</sup> It seems highly likely that concentration of 10  $\mu$ M was so high that morphine began binding to a pool of receptors that negatively affected the heart's tolerance to I/R. Gross's group was unable to protect hearts with 1  $\mu$ M BW373U86 in isolated murine hearts<sup>141</sup>

while BW373U86 did protect isolated chick cardiomyocytes but only at a concentration of 10 pM.<sup>107</sup> Mixing species always complicates interpretation but an obvious explanation is that overdosing can lead to negative off target effects. We recommend that *ex vivo* and *in vitro* experiments should test agonists at a concentration 10-fold higher than the  $K_i$  or  $EC_{50}$ .<sup>81</sup> They should also be aware that the binding affinities of these drugs can vary widely among species.

Aitchison *et al.* reported that DADLE at 10 nM exhibits infarct sparing that was diminished at 1  $\mu$ M. Inhibition of  $\kappa$  OR with nor-binaltorphimine restored the full cardioprotective effect of high concentration DADLE.<sup>142</sup> The authors concluded that the diminished effect of DADLE at high concentration is due to activate  $\kappa$  OR. These data closely resemble our results with DPDPE.<sup>104,112</sup> In addition, Aitchison *et al.* established that the non-selective  $\kappa$  OR agonist bremazocine (30 nM) *ex vivo* increased infarct size.<sup>142</sup> This negative effect of bremazocine disappeared after inhibition of  $\kappa$  OR. In this regard it should be noted that U50,488 is the selective  $\kappa_1$  OR agonist but bremazocine is an agonist of  $\kappa_2$  OR.<sup>143</sup> It seems reasonable to assume that the activation of  $\kappa_2$  OR exacerbates injury from I/R.

In 2005, Meine *et al.* published the results of a prospective, nonrandomized study, which included patients with acute coronary syndrome (ACS) with non-ST-segment elevation (NSTEMI) (n = 57,039).<sup>144</sup> The authors evaluated the outcome of patients treated with morphine and those who were not. It was found that treatment with morphine was associated with increased risk of in-hospital mortality. The authors raised concerns about the safety of using morphine in patients with ACS NSTEMI but pointed out that that could only be answered with a randomized trial.<sup>144</sup> These data were in accord with a few other studies.<sup>130,145-146</sup> In particular, Conahan *et al.* demonstrated that morphine (2 mg/kg) caused severe hypertension and an increase in systemic vascular resistance in patients undergoing heart valve replacement.<sup>146</sup> Later Lappas *et al.* reported that the addition of 5% NO to morphine (2 mg/kg intravenously) decreased blood pressure, cardiac index, stroke index and increased pulmonary capillary

wedge pressure.<sup>146</sup> Intravenous administration of morphine at a dose of 2.1 mg/kg can induce ST segment depression in patients with ischemic heart disease.<sup>130</sup> However, we would like to draw readers' attention to the fact that an extremely large dose of morphine (2 mg/kg) was used in these 3 studies.<sup>130,145-146</sup> Indeed, in current cardiological guidelines, the recommended dose of morphine is 4 - 8 mg (0.05 - 0.1 mg/kg).<sup>147</sup> Experimental studies suggest that morphine has the infarct-limiting effect at a dose of 0.3 mg/kg.<sup>10</sup> It comes as no surprise that morphine can provide an adverse effect on the cardiovascular system at a dose many times exceeding the therapeutic dose. In cardiological practice, morphine and fentanyl are used not only for pain relief in patients with AMI but also to ease anxiety, reduce preload, due to venodilation,<sup>147-149</sup> and afterload, due to reducing systemic vascular resistance.<sup>148,150</sup> It has been established that morphine and fentanyl can decrease myocardial oxygen consumption and reduce lactate production by the left ventricle in human.<sup>151</sup> Both of those effects may increase cardiac resistance to ischemia and improve the outcome in AMI. Morphine is also used for the prevention of pulmonary edema, and cardiogenic shock.<sup>152</sup> Therefore, morphine and other opioids are prescribed in the most serious cases characterized by a higher mortality than in patients with mild AMI, like those included in the study of Meine *et al.*<sup>144</sup> An overdose of opioids may cause a depression of respiration, hypotension and vomiting.<sup>152</sup>

In summary, pretreatment with agonists of  $\mu$ ,  $\delta_1$ ,  $\delta_2$  and  $\kappa_1$  OR exhibit cardioprotective properties both *in vivo* and *in vitro*. These pharmacological agents mimic the preconditioning phenomenon. The role of the ORL1-receptors in this regard remains open, however. A number of reports points to the existence of important species differences in the reaction of infarcted myocardium to opioids. Some receptors, such as  $\kappa_2$  ORs, may actually exacerbate the ischemic and reperfusion heart injury. But the agonists of  $\mu$ ,  $\delta_1$ ,  $\delta_2$  and  $\kappa_1$  ORs can be considered as the most promising group of agents able to induce cardioprotection. This opinion can be supported by numerous studies.<sup>80,87,93,96,98,106,109,110,115,116,121,128,129</sup>

## 5. Anti-apoptotic Effect of the Opioid Receptor Agonists

It is well known that reperfusion induces enhancement of production of reactive oxygen species (ROS)<sup>153</sup> and  $\text{Ca}^{2+}$  overload in cardiomyocytes.<sup>154</sup> Calcium ions and ROS evoke opening of MPT (mitochondria permeability transition pore) which is a protein supramolecular complex built into the outer and inner mitochondrial membrane.<sup>155,156</sup> Opening of this pore collapses the potential across the inner mitochondrial membrane, which prevents ATP generation by the mitochondria. MPT also releases cytochrome c and AIF (apoptosis inducing factor) in the intermembrane space into the cytosol.<sup>155,156</sup> Cytochrome C together with APAF-1 (apoptosis protease activating factor), procaspase-9 and ATP form a supramolecular complex named apoptosome.<sup>156</sup> The apoptosome catalyzes proteolysis of procaspase-9 to become active caspase-9, which in turn catalyze the cleavage of other proteins ultimately leading to apoptosis, a process in which the cell is killed and digested from within over several days. Protein AIF activates translocation of endonuclease G from cytosol into nucleus where the latter catalyzes DNA fragmentation that is a characteristic of apoptotic cells.<sup>156</sup> These events are developed mainly during the first minutes of reperfusion. Therefore, the opening of MPT is a major cause of death of cardiomyocytes after the restoration of coronary blood flow.<sup>155</sup> Necrosis quickly ensues if too many mitochondria within the cell are lost to MPT and the cell becomes tetrazolium negative (popular marker for infarct size studies) minutes after reperfusion due to membrane failure. If only a small fraction of the mitochondria are involved, however, the cell may survive the initial I/R only to succumb to apoptosis a day or two later. Apoptotic cells are tetrazolium positive in the first hours of reperfusion. The evidence is strong that IP protects by inhibiting MPT formation at reperfusion.<sup>157</sup> Generally, apoptosis and necrosis act in parallel and markers of apoptosis can be used to assess injury from I/R.

The first work indicating that opioids inhibit apoptosis of cardiomyocytes was published in 2001. Isolated chicken embryo cardiomyocytes were subjected to 12-h of hypoxia and 12-h of reoxygenation. Apoptosis was evaluated by the number of TUNEL-positive cells (terminal deoxyribonucleotide transferase-mediated dUTP nick end labeling). Fifty four % of the cells were TUNEL-positive. However, if BW373U86 (20 pM) was added to medium only 39 % of the cells became apoptotic. The selective inhibition of  $\delta_1$  OR with BNTX abolished the cytoprotective effect of BW373U86.<sup>158</sup> Okubo *et al.* found that opioids can exert an anti-apoptotic effect *in vivo*. Morphine at 0.3 mg/kg prior to coronary artery occlusion reduced the number of TUNEL-positive cells in the heart from 12.4% in control to only 5.2%. The  $\delta$  OR antagonist naltrindole (10 mg/kg intravenously) abolished the effect of morphine.<sup>159</sup> These findings led the investigators to conclude that the anti-apoptotic effect of morphine was dependent upon  $\delta$  OR activation. The anti-apoptotic effect of morphine on isolated cardiomyocytes was confirmed in later experiments.<sup>160</sup> In isolated perfused rat hearts exposed to 30 min global ischemia and 60 min reperfusion 16% of the cells were TUNEL-positive but with 3  $\mu$ M morphine in the perfusion solution decreased this index to 5%.<sup>161</sup> In 2012, Kim *et al.* using isolated cardiomyocytes found that addition of remifentanyl to the cell incubation medium prior to hypoxia/reoxygenation increased cell survival, decreased the concentration of  $\text{Ca}^{2+}$  in the cytoplasm, decreased activity of caspase-3, increased anti-apoptotic protein Bcl-2 (B-cell lymphoma protein-2) over that in untreated cells.<sup>162</sup> In 2009, it was noted that the selective  $\kappa_1$  OR agonist U50,488 causes an anti-apoptotic effect.<sup>163</sup> This study was performed in rats with coronary artery occlusion (45 min) and reperfusion (3 h). The  $\kappa_1$  OR agonist U50,488 was administered intravenously prior to ischemia. The number of TUNEL-positive cells in the area of I/R was 21.3% but in animals receiving U50,488 this number dropped to 12%. The selective  $\kappa$  OR antagonist nor-binaltorphimine eliminated this



effect indicating that the anti-apoptotic effect of U50,488 was mediated via  $\kappa_1$  OR activation. We recently confirmed their hypothesis using Q-U50,488, which does not cross the BBB.<sup>123</sup>

The aforementioned studies suggest that  $\delta$  and  $\kappa_1$  OR activation reduces the appearance of apoptosis of cardiomyocytes following reperfusion. It has not been determined whether agonists of  $\mu$  OR and ORL1 receptors can prevent apoptosis of cardiomyocytes.

## 6. Opioids Can Mimic Delayed Ischemic Preconditioning

Fryer *et al.* found that 24 h after injection of TAN-67, there was a return of protection against I/R. Combining TAN-67 with the  $\delta_1$  OR antagonist BNTX abolished this delayed protection. The authors concluded that the delayed protective effect of TAN-67 is dependent upon  $\delta_1$  OR activation.<sup>164</sup> The delayed protective effect of TAN-67 was confirmed in later works.<sup>165,166</sup> In 2004, it was found that the non-peptide  $\delta$  OR-selective agonist SNC-121 caused a delayed window of protection in rats and surprisingly its protection was retained after inhibition of ORs with naloxone.<sup>167</sup> The authors concluded that the cardioprotective effect of SNC-121 was not dependent on OR and illustrates the importance of testing with antagonists. Shinmura and colleagues found that the selective  $\delta$  OR agonist BW-373U86 can mimic delayed preconditioning.<sup>168</sup> Other investigators found that the non-peptide  $\delta_1$  and  $\delta_2$  OR agonist ARD-353 (0.3 mg/kg) evoked delayed conditioning.<sup>115</sup> Morphine (3 mg/kg) also triggered delayed conditioning<sup>169</sup> as did morphine at a dose of 0.3 mg/kg.<sup>170</sup> OR antagonists were not used in these two studies. Hence, the responsible for delayed protective effect OR was not identified.

A 30-min incubation of isolated cardiomyocytes with U50,488 for 20 h prior to hypoxia/reoxygenation increases cell tolerance to hypoxia/reoxygenation.<sup>171</sup> This effect of U50,488 did not occur after  $\kappa$  OR inhibition with nor-binaltorphimine. The delayed preconditioning phenomenon of U50,488 was confirmed in later works by the same

authors.<sup>172,173</sup> Intravenous administration of remifentanyl, a non-selective OR agonist, can induce a delayed cardioprotective effect<sup>174</sup> and this was confirmed by other investigators.<sup>175</sup> All three OR antagonists (CTOP, nor-binaltorphimine, naltrindole) abolished infarct-sparing effect remifentanyl.<sup>174</sup> Participation of ORs in the delayed cardioprotective effect of remifentanyl has been confirmed by Sun *et al.*<sup>175</sup>

## **7. Involvement of endogenous opioids in the infarct-reducing effect of remote ischemic preconditioning**

In 2001, Dickson *et al* attempted to clarify the nature of the humoral factor(s) mediating the infarct-reducing effect of remote ischemic preconditioning (RIPC).<sup>176</sup> Preconditioning of isolated perfused rabbit hearts was reproduced by three 5-min episodes of ischemia interspersed with 10 min of reperfusion. Coronary effluent was collected, purified and concentrated using Sep-Pak C-18 columns. They demonstrated that concentrated coronary effluent introduced to other isolated rabbit hearts can protect these hearts against ischemia (40 min) and reperfusion (120 min). This protective effect was eliminated by pretreatment with naloxone.<sup>176</sup> In the next study, isolated jejunal segments were subjected to 1 h of simulated ischemia followed by 30 min of reoxygenation.<sup>177</sup> Pretreatment with coronary effluent concentrate also improved contraction of the jejunal segments during reperfusion. Naloxone abolished the inotropic effect of the coronary effluent. Authors believe that coronary effluent contains opioids, which mediate a protective effect of RIPC.<sup>177</sup> The authors hypothesized that the endogenous mediator of the cardioprotective action of RIPC is endogenous opioid peptide Met5-enkephalin-Arg6-Phe7.<sup>178</sup> Patel *et al.* hypothesized that mesenteric preconditioning evokes release of endogenous opioids that protect the heart against I/R.<sup>179</sup> Rats were subjected to coronary artery occlusion (30 min) followed by reperfusion (2 h). Experimental groups underwent occlusion of the mesenteric artery (15 min)

followed by reperfusion (10 min). Pretreatment with naloxone abolished the protective effects of RIPC.<sup>179</sup> These data indicate that mesenteric preconditioning evokes release of endogenous opioid peptides that protect the myocardium against I/R. Weinbrenner *et al.* assumed that infarct-sparing effect mediated by infrarenal occlusion of the aorta (IOA) may be transmitted by endogenous opioids.<sup>180</sup> They established that IAO induced protected against I/R and this was abolished by pretreatment with the selective  $\delta 1$  OR antagonist BNTX (7-benzylidenenaltrexone).<sup>180</sup> These results indicate that the protection by RIPC is transmitted by  $\delta 1$  OR occupancy. Another group induced RIPC in rats by three cycles of femoral artery occlusion (5 min) followed by reperfusion (5 min).<sup>181</sup> They demonstrated that RIPC evokes increase in plasma dynorphin (a non-selective  $\kappa$  OR agonist), but not met-enkephalin (a  $\mu$  OR and  $\delta$  OR agonist) level. Pretreatment with the selective  $\kappa$  OR antagonist nor-binaltorphimine eliminated the infarct-sparing effect of RIPC. The selective  $\delta$  OR antagonist naltrindole had no effect on the remote preconditioning.<sup>181</sup> Hence, endogenous  $\kappa$  OR agonists, apparently dynorphin, mediate the cardioprotective effect of RIPC. Later, Rehmi *et al.* reported the participation of endogenous opioids in RIPC.<sup>182</sup> Rentoukas *et al.* reported that morphine in combination with RIPC reduced infarct size in patients with primary percutaneous coronary intervention while RIPC alone did not.<sup>183</sup>

Thus, today, there is no doubt that endogenous opioid peptides participate in the mechanism of the cardioprotective effect of RIPC. However, it remains unclear what kinds of ORs are involved in the RIPC phenomenon. The aforementioned Met5-enkephalin-Arg6-Phe7 and dynorphin are unlikely mediators of RIPC since they are not resistant to enzymatic hydrolysis.<sup>184,185</sup>

## 8. Opioids Mimic Postconditioning Phenomenon

The aforementioned studies demonstrate the ability of opioids to protect when applied as a pretreatment. A major indication for cardioprotection is acute coronary syndrome where the patient presents with ischemia already in progress. That makes pretreatment impossible so a postconditioning drug intervention is needed. Because much of the cell death in the heart is from MPT that form at reperfusion it is theoretically possible to protect against infarction right up to the time of reperfusion. IPost has been shown to limit infarct size.<sup>7</sup> Is it possible that OR agonists at reperfusion might also protect?

There are a few publications that indicate that the OR agonist can protect when administered at the end of the ischemic period. In one such study, rats were exposed to 1 h coronary artery occlusion and 2 h reperfusion. When 0.3 mg/kg morphine was administered intravenously 10 min prior to reperfusion it evoked a decrease in the IS/AAR from 45% to 30%.<sup>186</sup> ARD-353 administered after 30 min of ischemia at a dose of 0.3 mg/kg immediately before removing the ligature decrease the IS/AAR from 55% to 35%.<sup>115</sup> Since ARD-353 does not penetrate the BBB, these authors concluded that its infarct-reducing effect is mediated via peripheral OR.<sup>115</sup> Tsutsumi *et al.* studied mice with 30 min coronary artery occlusion and 2 h reperfusion. The  $\delta$  OR agonist SNC-121 (10 mg/kg) was administered intravenously 3 min before reperfusion. The control IS/AAR was 44% but only 24% in SNC-12-treated mice.<sup>187</sup> This study did not evaluate the role of OR antagonists.

The OR agonists mimic IPost not only *in vivo* but also *ex vivo*. In one study, the isolated perfused rat heart was exposed to 45 min global ischemia and 60 min reperfusion.<sup>136</sup> Morphine was added to the perfusion buffer at 0.3, 3, and 30  $\mu$ M for the first 10 min of reperfusion. Necrosis was assessed by tetrazolium staining and by CK-MB in coronary effluent. Morphine decreased the IS/AAR at 0.3  $\mu$ M and more so at 30  $\mu$ M. Pretreatment with naloxone or nor-binaltorphimine attenuated the protection.<sup>136</sup> Unfortunately, the OR

antagonists (naloxone, naltrindole, and nor-binaltorphimine) exerted a small but significant cardioprotective effect by themselves which complicates the interpretation.

In 2008, Jang *et al.* studied isolated rat heart with 30 min coronary artery branch occlusion and 2 h reperfusion. Either morphine (1  $\mu$ M) or the  $\delta$  OR agonist BW373U86 (1  $\mu$ M) were added to the perfusion solution starting 5 min prior to reperfusing the occluded coronary branch. The total duration of perfusion with agonists was 15 min. Both agonists decreased the IS/AAR by 3 fold.<sup>188</sup> Pretreatment with naltrindole (100  $\mu$ M) abolished the infarct-sparing effect of both agonists. Unfortunately, the authors used naltrindole in a concentration sufficient to inhibit all OR subtypes.<sup>135</sup> Using isolated perfused rat heart, Mourouzis *et al* reported that 10  $\mu$ M morphine can mimic IPost.<sup>189</sup> The ability of morphine at 1  $\mu$ M to postcondition was also reported elsewhere.<sup>190,191</sup> *In vivo* I/R experiments in rats showed that intravenous administration of U50,488 (0.1 mg/kg) 5 min prior to reperfusion promotes a decrease in the IS/AAR but U50,488 10 sec prior to reperfusion had no effect on the IS/AAR.<sup>192</sup> They also studied U50,488 (100 nM) in an isolated murine heart. The  $\kappa$  OR agonist was added to Krebs-Henseleit buffer at the beginning of reperfusion and it decreased the IS/AAR.<sup>192</sup> They did not test OR antagonists. However, this does not invalidate their conclusion that U50,488 protected via  $\kappa_1$  OR because  $K_i$  of U50,488 for  $\kappa_1$  OR is 7.4 nmol but the  $K_i$  of U50,488 for  $\mu$  OR is 256 nmol.<sup>134</sup>

Methadone administered to an in situ rat experiencing 30-min ischemia at a dose of 0.3 mg/kg for 5 min prior to reperfusion reduced infarct size. But if the injection was performed 10 sec after removal of the ligature, no changes in the IS/AAR could be detected.<sup>96</sup> If the duration of ischemia of the heart was 45 min, the injection of methadone 5 min before reperfusion also had no effect on the IS/AAR. The authors concluded that this opioid mimics IPost if it is administered 5 min before reperfusion.<sup>96</sup> Remifentanyl was infused intravenously for 5 min starting 5 min before reperfusion in rats with 30 min coronary artery occlusion and

2 h reperfusion. The IS/AAR was reduced by a dose of 10 µg/kg. Blocking δ or κ OR but not the µ OR by the agonist CTOP eliminated the protection.<sup>193</sup> The ability of remifentanyl to simulate IPost phenomenon was confirmed in another study performed in the isolated perfused rat heart.<sup>194</sup>

In 2011, it was reported that 1 µg/kg of a tetrapeptide referred to by the authors as Eribis peptide 94 (EP94) decreased the IS/AAR in rats at reperfusion.<sup>195</sup> These authors did not confirm a role of ORs in the infarct-reducing effect of EP94 but the authors did note that EP94 is a µ and δ OR agonist. Such a high potency of EP94 is surprising. However, in a later study by the same authors, it was reported that EP94 had an infarct-sparing effect at a dose of 25 µg/kg but had no effect on the infarct size at the dose of 1 µg/kg.<sup>196</sup> A 2012 study indicated that sufentanil simulates the IPost phenomenon at a dose of 1 µg/kg.<sup>197</sup> It is known that sufentanil is also a selective agonist of µ OR.<sup>198</sup> A further increase in the dose of this opioid did not lead to an enhancement of the infarct-reducing effect.<sup>197</sup> These data were confirmed in a later paper by the same group.<sup>199</sup> Unfortunately, these researchers did not test OR antagonists, therefore, it remains unclear whether the cardioprotective effect of sufentanil is depended upon µ OR activation. Most recently, in the experiments on isolated perfused rat heart, the non-selective OR agonist remifentanyl at reperfusion was protective.<sup>200</sup> The infarct-reducing effect of this opioid was eliminated by naloxone but the investigators did not use any of the selective OR antagonists.

Thus, the aforementioned studies provide ample evidence that activation of δ and κ<sub>1</sub> OR can postcondition the heart. It remains unclear whether agonists of µ OR and ORL1 are also protective at the time of reperfusion.

## **9. Localization of Opioid Receptors that Protect the Heart from Ischemia/Reperfusion**

Studies on the isolated heart seem to indicate that the infarct-limiting effect of opioids is associated with the occupancy of the cardiac ORs. However, one should pay attention to two facts: (1) most studies have used OR ligands that penetrate the BBB; (2) in some studies the OR agonists were used in very large doses.<sup>81,91,187</sup> For example, TAN-67 was used at a dose of 10 mg/kg.<sup>91</sup> But according to Knapp *et al.* the  $K_i$  of TAN-67 for  $\delta$  OR is 0.65 nmol.<sup>201</sup> For comparison, the  $K_i$  of morphine against  $\delta$  OR is 49 nmol.<sup>135</sup> One can assume that in order to limit the size of myocardial infarction, a larger dose of morphine would be required. However, it has been demonstrated that morphine is protective at a dose of only 0.3 mg/kg in rats.<sup>9</sup> An interesting possible explanation of this paradox could be that TAN-67 activates a central  $\delta$  OR that remotely increases cardiac tolerance to I/R via neural pathways and the low penetration of the BBB for TAN-67 requires a higher dose.

There is a direct evidence of participation of central ORs in cardioprotection. A rat study with 30 min coronary artery occlusion and 90 min reperfusion showed that intrathecal administration of morphine (0.3  $\mu$ g/kg) for 20 min prior to ischemia promotes a decrease in the IS/AAR.<sup>202</sup> In a similar study in 2009, intrathecal pretreatment with morphine again protected rat hearts in a dose-dependent manner.<sup>203</sup> Intrathecal administration of CTOP, naltrindole or nor-binaltorphimine abolished the infarct-sparing effect and the authors concluded that all three ( $\mu$ ,  $\delta$ , and  $\kappa$ ) ORs are involved in the cardioprotective effect of morphine.<sup>203</sup> The infarct-reducing effect of morphine during intrathecal administration was confirmed in 2010.<sup>204</sup> A cardioprotective effect of morphine was seen when it was infused for the 5 min prior to reperfusion and could be blocked by inhibition of  $\mu$ ,  $\delta$  or  $\kappa$  OR.<sup>205</sup>

In a study in rats with coronary artery occlusion/reperfusion, morphine was administered intravenously at a dose of 0.3 mg/kg.<sup>84</sup> Naloxone methiodide, which does not cross the BBB, was administered prior to morphine injection intravenously or intrathecally at a dose of 20 mg/kg or 20  $\mu$ g/kg.<sup>84,206</sup> Regardless of the route of administration, naloxone

methiodide abolished the infarct-sparing effect of morphine. The authors concluded that morphine protected through both central and peripheral ORs.

In 2012, it was shown that intrathecal administration of morphine to rats decreased in the IS/AAR by twofold and the autonomic ganglion blocker hexamethonium completely abolished the protection.<sup>207</sup> They concluded that the cardioprotective effect of morphine was mediated via central OR stimulation and signaling through the autonomic nervous system. In 2014, it was found that intrathecal administration of  $\mu$  OR agonist fentanyl evokes a decrease in the IS/AAR.<sup>208</sup> These data also indicate that the infarct-reducing effect of opioids following intravenous administration may not only be a consequence of activation of peripheral but also of central ORs. The infarct-limiting effect of opioid peptide EP94 occurred after blockade of peripheral OR with naloxone methiodide but disappeared after blocking peripheral and central ORs with naloxone.<sup>129</sup> These authors concluded that the infarct-reducing effect of EP94 is mediated via central OR activation. This result was surprising because opioid peptides usually penetrate the BBB poorly. For example, the opioid peptide dalargin exerts central effect only at a dose of 500  $\mu\text{g/kg}$ .<sup>209</sup> But Gross *et al.* used EP94 at a dose of 1  $\mu\text{g/kg}$ .<sup>129</sup>

Thus, central OR stimulation clearly can increase cardiac tolerance to I/R. On the other hand, there is ample data with isolated hearts that cardiac OR can also protect the heart. It remains unclear, therefore, to what extent the infarct limiting effect of opioids during intravenous administration is mediated via central OR activation.

### **10. Effect of Opioids on Recovery of Cardiac Contractility During Reperfusion**

The above studies primarily concentrated on myocardial necrosis as the endpoint. Cardiac injury also manifests itself as a reduction in post-reperfusion cardiac contractility. That reduction can be from loss of muscle to necrosis or it can be due to stunning, which is a transient loss of contractility following I/R. Preservation of mechanical function after I/R is



paramount in the setting of cardiac surgery. Therefore, some studies used cardiac contractility as their endpoint rather than infarction.

#### **A. $\mu$ OR**

We found that intravenous administration of the  $\mu$  OR-selective agonists DALDA (0.1 mg/kg) or DAMGO (1 mg/kg) for 15 min prior to heart isolation promotes better recovery of ventricular developed pressure (LVDP) after I/R in the isolated rat heart. The  $\mu$  OR-selective antagonist CTAP (0.1 mg/kg) completely abolished DAMGO's protective effect. In contrast, perfusion of the isolated rat heart with DAMGO (0.1 mg/L or 195 nM) for 10 min prior to ischemia did not improve recovery of function.<sup>127</sup> Only activation of the  $\mu$  OR *in vivo* preserves post-ischemic contractility *ex vivo*. It is known that  $K_i$  of DAMGO for  $\mu$  OR is 1.23 nmol<sup>110</sup> or 27 nmol<sup>210</sup>. Therefore, we can't explain an absence of inotropic effect of DAMGO *ex vivo* by a too low concentration of peptide. The protective effect of the  $\mu$  OR agonist must be dependent upon  $\mu$  OR activation somewhere outside the heart.<sup>47,64,211</sup>

#### **B. $\kappa$ OR vs $\delta$ OR**

In a study on isolated rat heart, it was seen that perfusion with 200  $\mu$ M DADLE prior to hypothermic cardiac arrest decreases the post-ischemic rise in end diastolic pressure (EDP) but not the decline in left ventricular developed pressure (LVDP).<sup>92</sup> In 1999, P.E. Benedict *et al.* subjected the isolated rabbit heart to cardioplegic arrest (2 h of 34°C ischemia) followed by reperfusion. Perfusion of the isolated heart with morphine prior to ischemia promotes an increase in contractility during reperfusion.<sup>212,213</sup> The selective  $\mu$  OR agonist fentanyl<sup>198</sup> did not have a similar effect. Consequently, it may be concluded that positive inotropic effect of morphine was depended upon  $\delta$  or  $\kappa$  OR activation. The British physiologists Kato and Foex subjected the isolated perfused rat heart to 30 min global ischemia and 60 min reperfusion. The heart was perfused with the  $\mu$  OR agonist fentanyl (740 nM). Fentanyl increased LVDP, the rate of contraction and the rate of relaxation of heart during reperfusion and pretreatment

with naloxone abolished fentanyl's protective effect. These authors concluded that the inotropic effect of fentanyl was dependent upon  $\delta$  OR activation.<sup>214</sup> Kato and Foex gave fentanyl at a concentration sufficient to occupancy  $\mu$ ,  $\delta$  and  $\kappa$  OR.<sup>214</sup> Therefore, in our opinion, the presented data do not allow one to make a conclusion that the protective effect of fentanyl is mediated via  $\delta$  OR stimulation. To further complicate the issue, the same authors published a paper in 2000, which reported that there was no improvement of contractility in the reperfusion period after 740 nM fentanyl.<sup>215</sup> It is unclear, which study is correct.

Exposing the isolated rat heart to the selective  $\kappa_1$  OR agonist U50,488 (1  $\mu$ M) for 2 min starting 10 min prior to global I/R promoted an increase in LVDP in the reperfusion period.<sup>216</sup> The  $\kappa_1$  OR agonist had no effect on the EDP when given only during reperfusion. The inotropic effect of U50,488 was blocked by pretreatment with the selective  $\kappa$  OR antagonist nor-binaltorphimine (1  $\mu$ M during 4 min). These authors concluded that the protective effect of U50,488 was dependent upon  $\kappa$  OR activation. Their work could be criticized because they did not use U50,488 and nor-binaltorphimine at receptor-selective doses. The  $K_i$  of U50,488 for  $\kappa_1$  OR is 0.89 nmol<sup>217</sup> and the  $K_i$  of nor-binaltorphimine for  $\kappa$  OR is 0.18 nmol.<sup>218</sup> Nor-binaltorphimine at the final concentration of 100 nmol will also inhibit  $\delta$  OR.<sup>219</sup> In 2001, Genade *et al.* found that perfusion of the isolated heart with 10 nmol DADLE prior to ischemia improved mechanical function after reperfusion.<sup>220</sup> Since DADLE at 10 nmol should only interact with  $\delta$  OR<sup>221</sup>, it may be assumed that the inotropic effect of DADLE was dependent upon  $\delta$  OR activation.

We perfused isolated rabbit heart with 2 mM DADLE for 15 minutes before cardioplegic arrest and a 2-h global ischemia followed by reperfusion.<sup>222</sup> DADLE promoted an increase in LVDP over those hearts which were subjected to only cardioplegia.<sup>222</sup> DADLE at the concentration of 2 mM activates all ORs.<sup>221,223</sup> Therefore, it is not clear what OR subtype was involved in the protective effect of DADLE. We continued this study with swine

hearts. After pretreatment with DADLE (1 mg/kg intravenously), morphine (1 mg/kg intravenously), or saline, hearts were excised and kept for 75 minutes at 4°C, then reperfused them in a four-chamber isolated working heart apparatus.<sup>224</sup> We found that pretreatment with either DADLE or morphine promoted an increase in cardiac output during reperfusion. Since neither DADLE nor morphine are the  $\delta$  OR-selective agonists, it remains unclear what OR subtype was involved. We suspect that the protective effect of DADLE was dependent upon  $\mu$  OR stimulation as noted in another of our studies.<sup>127</sup> Similar data were obtained by Shinmura *et al.* They injected the  $\delta$  OR-selective agonist BW-373U86 (1 mg/kg) into rats subcutaneously either for 1 h or 24 h before the heart isolation. Isolated perfused rat hearts were subjected to 20 min of global ischemia followed by 20 min of reperfusion. Pretreatment with BW-373U86 improved LVDP during reperfusion.<sup>225</sup> Such evidence indicated that BW-373U86 mimics both preconditioning and delayed preconditioning. It was not determined as to what OR subtype(s) were involved.

In 2002, Wu *et al.* subjected isolated perfused rat hearts to 30 min global ischemia and 2 h reperfusion. Hearts were perfused with 100 nM [Dmt<sup>1</sup>]DALDA or 1  $\mu$ M morphine for 30 min and then subjected to 30-min global ischemia. Reperfusion was performed using the same solutions. Both opioids increased contractile force during reperfusion over that seen with buffer only. The protection was present even when hearts were only perfused with [Dmt<sup>1</sup>]DALDA during reperfusion, whereas reperfusion with morphine only during reperfusion had no effect on the contractility.<sup>226</sup> It is known that the peptide [Dmt<sup>1</sup>]DALDA is an agonist of  $\mu$  and  $\kappa$  OR.<sup>227</sup> Therefore, it remains open as to what OR was involved.

Peart and Gross presented evidence that either  $\delta$  or  $\kappa$  OR stimulation improves cardiac function during reperfusion.<sup>141</sup> Isolated murine heart was subjected to 20 min global ischemia followed by 45 min reperfusion. The OR agonists were infused for 10 min prior to ischemia, and then throughout reperfusion. Infusion of 10  $\mu$ M morphine induced an improvement in

post-ischemic recovery. Infusion with the selective  $\delta$  OR agonist BW373U86 (1  $\mu$ M) also improved recovery of LVDP. Pretreatment with the selective  $\delta_1$  OR antagonist BNTX (1  $\mu$ M) completely abolished this effect of BW373U86. Infusion of the selective  $\kappa_1$  OR agonist U50,488 (1  $\mu$ M) produced a marked improvement in contractile recovery.<sup>141</sup> This effect was blocked by the selective  $\kappa$  OR antagonist nor-binaltorphimine (1  $\mu$ M).

Gross's group showed that pretreatment with the selective  $\delta$  OR agonist DPDPE (1  $\mu$ M) also improves mechanical recovery of murine hearts following ischemia.<sup>228</sup> In another study, cardioplegic arrest during global ischemia (2 h at 34°C) was induced and followed by reperfusion. Hearts which were pretreated with either the preferential  $\delta$  OR agonist DADLE or the  $\kappa$  agonist OR U50,488 demonstrated significantly improved functional recovery versus controls. The selective  $\mu$  OR agonist fentanyl had no effect on recovery.<sup>229</sup> Selective antagonists were not tested. An improvement of contractility during reperfusion after U50,488 was confirmed in isolated rat hearts.<sup>230</sup> This effect was abolished after pretreatment with nor-binaltorphimine indicating the protective effect of U50,488 is mediated via  $\kappa$  OR occupancy. Perfusion of the isolated rat heart with a solution containing the nonselective  $\kappa$  OR agonist pentazocine before or after 15 min global ischemia improved cardiac contractility during reperfusion.<sup>231</sup> These authors did not test with the OR antagonists. It was also shown that preliminary perfusion of the isolated heart with 1  $\mu$ M morphine for 15 min before global ischemia promoted an increase in LVDP in the reperfusion period.<sup>161</sup> These authors also did not test any OR antagonist. It should be noted that some investigators did not find a positive effect of morphine or U50,488 on cardiac contractility although they did decrease infarct size.<sup>194,232</sup>

## **11. Worsening of post-ischemic mechanical recovery by opioid ligands**

In the above studies, we presented data that the OR agonist can prevent an appearance of reperfusion contractile dysfunction. However, there are reports that some opioids can also exacerbate contractile dysfunction. The  $\kappa$  OR agonist bremazocine exacerbates reperfusion contractile dysfunction of the isolated heart.<sup>142</sup> It is known that bremazocine is a potent  $\kappa_2$  OR agonist.<sup>143</sup> Therefore, the above presented data on the effects of the  $\kappa_1$  OR agonist U50,488 and bremazocine do not contradict each other.

We observed that intravenous administration of the  $\delta_1$  OR-selective agonist DPDPE (0.1 or 0.5 mg/kg) 15 min prior to the heart isolation exacerbates reperfusion contractile dysfunction.<sup>112</sup> If we added 0.1 or 0.5 mg/L DPDPE (154 nM or 771 nM) 15 min before global ischemia (45 min) and reperfusion (30 min), we also observed exacerbation of contractile dysfunction. DPDPE peptide can interact with only  $\delta_1$  OR at the final concentration of 154 nM.<sup>198</sup> Pretreatment with the selective  $\delta$  OR antagonist naltrindole (1 nM) completely abolished the negative inotropic effect of DPDPE (154 nM).<sup>106</sup> We later found that the selective  $\delta_1$  OR agonist TAN-67 (178 nM) also exacerbates dysfunction during reperfusion.<sup>103</sup> Pretreatment with the selective  $\delta$  OR antagonist naltrindole (1 nM) abolished this effect of TAN-67. Our above result is drastically different from the data of Gross's group<sup>141,228</sup> where they generally found protection from  $\delta_1$  OR agonists. It worth mentioning that their schedule of drug administration was quite different from that used in the above studies, however.

In most of the above studies, the ischemic period was long enough to cause some necrosis of the heart. In those studies, the post-ischemic recovery is influenced by a combination of stunning and infarction; so it is not clear which was contributing to an enhanced post-ischemic improvement in mechanical function. This is important in that the mechanisms of the two forms of injury differ drastically. In 2006, Grosse Hartlage *et al.* employed a pure stunning model where a coronary branch of a chronically instrumented dog

is given a 10 min coronary occlusion, which is too short to cause any infarction but does depress post-ischemic function. Function completely recovers spontaneously in a day proving that the segment was only stunned. They gave the selective  $\kappa$  OR receptor antagonist norbinaltorphimine (2.5 mg/kg intravenously). Pretreatment with the  $\kappa$  OR blocker prevented the decrease in ventricular wall function after ischemia. They found evidence that the endogenous opioid dynorphin was elevated in the plasma after the ischemic insult and concluded that this opioid was exacerbating the dysfunction in the untreated dogs.<sup>233</sup> This data contradicts the abovementioned data on positive inotropic effect of the  $\kappa$  OR agonist U50,488 during reperfusion<sup>141,230</sup> but those studies used isolated hearts with long ischemic periods where the agonist was confined to the pretreatment period. We found that perfusion of the isolated rat heart with solution containing U50,488 (0.1  $\mu$ M) starting 10 min before global ischemia (45 min) decreases creatine kinase release during reperfusion but depresses the recovery of contractile dysfunction.<sup>120</sup> If we used U50,488 at the final concentration of 1  $\mu$ M, the cardioprotective effect disappeared but the negative inotropic effect was enhanced.

Thus, results of studies of the inotropic effects of opioids on cardiac stunning are very contradictory. Some studies indicated that pretreatment with opioids improves cardiac contractility in reperfusion period.<sup>92,127,161,213,214,216,220,222,225,226,230,231</sup> Other studies showed that pretreatment with opioids exacerbate contractile dysfunction.<sup>103,106,112,120,142</sup> Other investigators could not find any alteration of postischemic recovery after pretreatment with the OR agonists.<sup>127,194,214,232</sup> Much of this confusion no doubt arises from heterogeneity in the models (isolated vs. in situ), the schedule of drug administration (pretreatment vs. post treatment vs. continuous treatment), and the type of injury (infarction vs. stunning). Therefore, the resolution of possible inotropic effects of opioids during myocardial reperfusion remains to be determined.

## **12. Antiarrhythmic Effect of the Opioid Receptor Ligands**

The most frequent causes of death from myocardial infarction are cardiogenic shock (52%), arrhythmias (25%), thromboembolism of the pulmonary artery (10%), and rupture of the left ventricle (5%).<sup>234</sup> These findings indicate that an antiarrhythmic drug could dramatically reduce mortality in this population. Opioids are potential candidates for developing such drugs.

The first report that an OR agonist has an antiarrhythmic effect was with meptazinol during coronary artery occlusion in rats in 1983.<sup>235</sup> In 1989, it was shown that the selective  $\mu$  OR agonist fentanyl (60  $\mu\text{g/kg}$  intravenously) increased the ventricular fibrillation threshold (VFT) in dogs with coronary artery occlusion.<sup>236</sup> The  $\mu$  and  $\kappa$  OR agonist buprenorphine had the same effect.<sup>236</sup> The antifibrillatory activity of the  $\mu$  OR agonists fentanyl, sufentanil and carfentanil in dogs with coronary artery occlusion was demonstrated by Hess *et al.* in 1989.<sup>237</sup> Clinical observations established that fentanyl (60  $\mu\text{g/kg}$  intravenously) could prevent the appearance of intraoperative ventricular fibrillation during cardiosurgery intervention in neonates.<sup>238</sup> These studies indicate that opiates can increase cardiac tolerance to the arrhythmogenic effect of ischemia/reperfusion.

Unfortunately, these historical studies were performed before highly selective OR antagonists were widely available; so none of these publications contained this approach aimed to confirm a receptor-mediated effect and identify which subtype was responsible. Morphine is a  $\mu$  OR-selective agonist as is fentanyl.<sup>198</sup> However, it was later shown that the cardioprotective effects of morphine<sup>10</sup> and fentanyl<sup>214</sup> are actually dependent upon  $\delta$  OR stimulation. Furthermore, both narcotic analgesics easily penetrate through the BBB. Therefore, it was unclear whether their antiarrhythmic effect was dependent upon the central or peripheral OR occupancy.

#### **A. $\mu$ OR agonists**

In order to find out whether the peripheral ORs are involved in the arrhythmogenesis,

we used D-Ala<sup>2</sup>,Leu<sup>5</sup>,Arg<sup>6</sup>-enkephalin (dalargin). This compound can penetrate the BBB at a dose of 0.5 mg/kg and higher.<sup>209</sup> We found that intravenous administration of dalargin (0.1 mg/kg) decreases the incidence of ventricular fibrillation during coronary artery occlusion in rats.<sup>239</sup> Other investigators confirmed our data in experiments on cats.<sup>240</sup> According to the data of Grekova *et al.* dalargin (0.1 mg/kg) exhibits an antiarrhythmic effect when administered intravenously to dogs 5 min prior to coronary artery occlusion.<sup>241</sup> Dalargin can prevent both ischemic and reperfusion arrhythmias. However, this opioid peptide was ineffective if it was administered after coronary artery ligation.<sup>241</sup> Dalargin not only prevented the appearance of arrhythmias during ischemia, it also evoked an increase in the VFT in rats with postinfarction fibrosis.<sup>242</sup> We therefore reasoned that the antiarrhythmic effect of dalargin is mediated via peripheral OR activation. However, it is still unknown what OR subtypes are involved in antiarrhythmic effect of dalargin because this peptide is a  $\mu$  and  $\delta$  OR agonist.<sup>243,244</sup>

We found that the injection of the selective  $\mu$  OR agonist DALDA prior to a 10-min coronary artery occlusion and reperfusion in rats does not affect the incidence of ventricular arrhythmias.<sup>138</sup> Nor could we find an antiarrhythmic effect of the selective  $\mu$  OR agonist DMGO (150 or 1500 nmol/kg) or the  $\mu$  OR agonist dermorphin H (150 nmol/kg) during coronary artery occlusion.<sup>245</sup> In experiments with postinfarction cardiac fibrosis we obtained data that the nonselective  $\mu$  OR agonists morphine and dalargin and the  $\mu$  OR agonist DALDA increase the threshold for fibrillation.<sup>242,246,247</sup> The antifibrillatory effect of DALDA (0.1 mg/kg) was not present after inhibition of the peripheral ORs with naloxone methiodide.<sup>247</sup> We theorize that the antifibrillatory effect of DALDA is depended upon peripheral OR occupancy. Blockade with The  $\mu$  OR-selective antagonist CTAP (0.5 mg/kg) also abolished the antifibrillatory effect of DALDA.<sup>247</sup> Therefore, it can be argued that DALDA induces an increase in cardiac electrical stability via the peripheral  $\mu$  OR activation.



Thus, peripheral  $\mu$  OR activation increases cardiac electrical stability in animals with postinfarction cardiac fibrosis but, based on our data,  $\mu$  OR agonists do not suppress acute I/R arrhythmias.

***B.  $\delta$  OR and arrhythmias from acute ischemia/reperfusion***

In more recent experiments with the selective  $\delta_1$  OR agonists TAN-67 (0.08 mg/kg), DPDPE (0.1, 0.2 and 0.5 mg/kg) and the selective peptide agonist DSLET (0.11 mg/kg), we found that these ligands after intravenous administration had no effect on the incidence of ventricular arrhythmias during a 10-min coronary artery occlusion and reperfusion in rats.<sup>138</sup> However, we found that selective  $\delta_2$  OR activation with intravenous administration of deltorphin II (0.12 mg/kg or 150 nmol/kg) did reduce arrhythmias from I/R.<sup>138</sup> We suspect that the strong antiarrhythmic effect of deltorphin II and the absence thereof, in DSLET is a consequence of different affinities of these ligands to  $\delta$  OR. Deltorphin II exceeds DSLET in two-fold in its affinity for  $\delta$  OR.<sup>248</sup> Therefore, it is not surprising that when using both peptides in equimolar doses only deltorphin II was antiarrhythmic. Recently, we have shown the selective  $\delta_2$  OR agonist deltorphin II (150 nmol/kg) and the putative  $\delta_2$ -selective deltorphin D<sub>var</sub> (150 nmol/kg) and deltorphin E (150 nmol/kg) had antiarrhythmic properties during coronary artery occlusion/reperfusion.<sup>245</sup> We performed further studies on Deltorphin II, which exerted the most pronounced antiarrhythmic effect. Pretreatment with the nonselective OR antagonist naltrexone (5 mg/kg), the nonselective peripheral OR antagonist naloxone methiodide (5 mg/kg) or the selective  $\delta_2$  OR antagonist naltriben (0.3 mg/kg) completely abolished deltorphin II's antiarrhythmic effect. But pretreatment with the  $\delta_1$ -selective antagonist BNTX (0.7 mg/kg) did not abrogate deltorphin II's antiarrhythmic effect. Therefore, we concluded that peripheral  $\delta_2$  OR stimulation enhances cardiac tolerance to arrhythmogenic impact of ischemia and reperfusion.<sup>245</sup>

We found that intravenous administration of the  $\delta_1$  OR-selective peptide DPDPE at a

dose of 150 and 1500 nmol/kg had no effect on the incidence of ischemic and reperfusion ventricular arrhythmias in rats.<sup>245</sup> Most of the opioid peptides poorly penetrate the BBB.<sup>23,24,209</sup> We also tested the  $\delta_1$  OR-selective agonist TAN-67 and found that this opioid also was not antiarrhythmogenic.<sup>249</sup> Therefore, we concluded that peripheral  $\delta_1$  OR agonists are not antiarrhythmogenic. However, Fryer *et al.* demonstrated an antiarrhythmic effect of TAN-67 in open-chest rats.<sup>102</sup> Unlike our study, they gave TAN-67 at a dose 10 mg/kg (125 fold higher the 0.08 mg/kg dose used by us). The higher dose would have penetrated the BBB. The antiarrhythmic effect of TAN-67 was no longer seen after selective blocking of  $\delta$  OR.<sup>102</sup> We suggest that the antiarrhythmic effect of TAN-67 is mediated via central OR stimulation. We conclude that peripheral  $\delta_2$  OR activation increases cardiac tolerance to the arrhythmogenic effect of ischemia/reperfusion while occupancy of the central  $\delta_1$  OR seems to have the same effect.

### *C. $\delta$ OR and arrhythmias from postinfarction cardiac fibrosis*

The picture is different with arrhythmias generated by postinfarction cardiac fibrosis. Intravenous administration of the selective  $\delta_1$  OR agonist DPDPE (0.1 mg/kg) increased the fibrillation threshold by 36 % in rats with postinfarction cardiac fibrosis.<sup>250</sup> The antifibrillatory effect of DPDPE was lost after blockade of peripheral OR with naloxone methiodide or after selective inhibition of  $\delta$  OR with ICI 174,864. The selective  $\delta_2$  OR agonist DSLET (0.5 mg/kg) did not exert any antifibrillatory effect. In this study, we did not test selective  $\delta_1$  and  $\delta_2$  OR antagonists. Nevertheless, we suspect that the antiarrhythmic effect depended upon peripheral  $\delta_1$  OR stimulation.

## **13. Controversy over $\kappa$ OR and arrhythmias**

In 1992, it was found that the selective  $\kappa$  OR agonist U-50,488 (7.5 mg/kg, intravenously) enhanced cardiac tolerance to the arrhythmias from a 30-min coronary artery

occlusion. Because pretreatment with naloxone (2.5 mg/kg) did not eliminate U-50,488's effect, these authors concluded that the protection was not dependent upon activation of  $\kappa$  OR.<sup>251</sup> They proposed that the antiarrhythmic effect was caused by an off-target blockade of fast  $\text{Na}^+$  channels because after injection of U-50,488 they observed bradycardia and prolongation of the QRS duration (effects that are typical for I class antiarrhythmic drugs).<sup>251</sup> However, our results did not concur with these data. They gave U-50,488 at a dose of 7.5 mg/kg and naloxone was administered at a dose of only 2.5 mg/kg, a dose which is not high enough for inhibition of  $\kappa$  OR.<sup>198</sup> We found that a lower dose of U-50,488 (1 mg/kg intravenously) prevents ventricular arrhythmias during coronary artery occlusion and reperfusion.<sup>252</sup> We believe that the dose of U-50488 (7.5 mg/kg) used by Pugsley *et al.* was too high causing it to exhibit non-receptor effects.

The above researchers tried to compare antiarrhythmic properties of the  $\kappa$  OR agonist PD 129290 and its R,R (+)-enantiomer which has a low affinity to  $\kappa$  OR.<sup>253</sup> Both enantiomers at a dose of 3 mg/kg decreased arrhythmias from a 30-min coronary artery occlusion. Since, naloxone at a dose of 2.5 mg/kg did not abolish this effect, these authors concluded again that the antiarrhythmic effect of both enantiomers was independent of  $\kappa$  OR. Furthermore, both enantiomers increased the QRS duration and in isolated cardiomyocytes, both compounds did inhibit  $\text{Na}^+$  current.<sup>253</sup> They again concluded that the antiarrhythmic effect of these opioids is due to blocking  $\text{Na}^+$  channels. In a study with isolated cardiomyocytes, the same research group found that U-50488 and PD 129290 also inhibit  $\text{Na}^+$  current. The  $\kappa$  OR antagonist itself MR2266 did not produce any change in the  $\text{Na}^+$  or  $\text{K}^+$  currents, nor did it alter the channel blocking properties of U-50,488.<sup>254</sup> The electrophysiological effects of U-50,488 were compared with those of the class Ib antiarrhythmic agent lidocaine in rat heart and the sodium currents expressed in *Xenopus laevis* oocytes by using two-electrode voltage clamp.<sup>255</sup> Both U-50,488H and lidocaine produced a concentration-dependent tonic block of  $\text{Na}^+$  current but

U-50,488H was approximately 4 fold more potent than lidocaine. These authors maintain that the antiarrhythmic properties of the  $\kappa$  OR agonists do not depend on OR activation and is an outcome of nonspecific  $\text{Na}^+$  channel blocking.<sup>253-255</sup>

We continued the study using U-50488 enantiomers, which differ in affinity to  $\kappa_1$  OR.<sup>217</sup> It appeared that (-)-trans-(1S,2S)-U-50,488 (1 mg/kg intravenously) with high affinity to  $\kappa_1$  OR can reduce arrhythmias from 10 min of coronary artery occlusion and reperfusion in rats.<sup>252,256,257</sup> An enantiomer (+)-trans-(1R,2R)-U-50,488 with low affinity to  $\kappa_1$  OR did not exert similar effect.<sup>252,256,257</sup> Others have shown that the preferential  $\kappa_1$  OR agonist dynorphin  $\text{A}_{1-13}$  at a dose of 40  $\mu\text{g/kg}$  intravenously also exerts antiarrhythmic effect in cats with coronary artery occlusion.<sup>258</sup> Furthermore, we established that pretreatment with the selective  $\kappa$  OR antagonist nor-binaltorphimine (9 mg/kg, intravenously) completely abolished antiarrhythmic effect of (-)-U-50,488.<sup>256</sup> Pretreatment with the  $\kappa_2$  OR antagonist quadazocine (3 mg/kg, intravenously) did not alter antiarrhythmic effect of (-)-U-50,488.<sup>256</sup> The selective  $\kappa_2$  OR agonist GR-89696 (25  $\mu\text{g/kg}$ , intravenously) also had no effect on the reperfusion arrhythmias in rats.<sup>259</sup> However, inhibition of peripheral ORs with naloxone methiodide (5 mg/kg, intravenously) completely abolished antiarrhythmic effect of (-)-U-50,488.<sup>259</sup>

Comparison of the above observations convinced us that peripheral  $\kappa_1$  OR activation can enhance cardiac tolerance to ischemia/reperfusion-generated arrhythmias. The basis for this assertion are our data that the selective  $\kappa_2$  OR agonist GR-89696 does not exhibit antiarrhythmic properties and that the  $\kappa_2$  OR antagonist quadazocine did not abolish the antiarrhythmic effect of (-)-U-50,488.<sup>259</sup> But pretreatment with naloxone methiodide or nor-binaltorphimine does abolish the antiarrhythmic effect of (-)-U-50,488.<sup>259</sup> At high concentration, (-)-U-50,488 clearly does block sodium channels. But at a low dose, the antiarrhythmic effect of U-50,488 is mediated via  $\kappa_1$  OR occupancy alone.

We determined that intravenous administration of the selective ORL1 agonist

nociceptin at a dose of 220 or 1500 nmol/kg had no effect on the incidence of ischemic and reperfusion-induced ventricular arrhythmias *in vivo*.<sup>245</sup> However, nociceptin is not resistant to enzymatic hydrolysis. Therefore, we cannot completely exclude the possibility that a selective ORL1 agonist will exhibit antiarrhythmic properties.

#### 14. Arrhythmogenic and proarrhythmic effects of opioids

It has been shown that injection of the non-selective OR agonist  $\beta$ -endorphin into perfusion solution caused only atrial fibrillation and atrioventricular block in isolated rat heart.<sup>260,261</sup> It should be noted that *in vivo*  $\beta$ -endorphin exhibits antiarrhythmic properties in anesthetized cats with coronary artery occlusion.<sup>262</sup> In 1987, Lee and Wong demonstrated that injection of the preferential  $\kappa$  OR agonist dynorphin1-13 (20  $\mu$ g/heart) caused both atrial and ventricular arrhythmias in isolated rat heart.<sup>263</sup> This effect was antagonized by naloxone. In 1990, Wong et al published results of their experiments on isolated Langendorff-perfused rat heart.<sup>264</sup> The OR agonists and antagonists were injected directly into the aorta through cannulas. The OR antagonists were administered 1 min before the administration of OR agonist or 20 min global ischemia and 60 min reperfusion. The selective  $\mu$  OR agonist DAMGO evoked atrial arrhythmias and at a higher dose caused frequent premature ventricular contractions (PVC).<sup>264</sup> The selective  $\kappa$  OR agonist U50488 caused both atrial and ventricular arrhythmias. At a high dose (132 nmol/heart), this opioid induced frequent PVC and ventricular tachycardia. The  $\delta$  OR agonists DPDPE and DADLE evoked only atrial arrhythmias. The arrhythmogenic effects of U50488 were attenuated by pretreatment with the  $\kappa$  OR antagonist MR 2266 in a dose-related manner whilst the proarrhythmic effect of DAMGO was abolished by the preferential  $\mu$  OR antagonist naloxone. Naloxone itself exhibited a weak antiarrhythmic effect manifested in prevention of only ventricular tachycardia during reperfusion. Authors concluded that the cardiac  $\kappa$  OR are the most likely

receptors involved in arrhythmogenesis during ischemia and reperfusion.<sup>264</sup> The limitation of this study was the fact that the bolus administration of opioids did not allow them to compare the concentrations of the opioids with their respective K<sub>d</sub>s and K<sub>i</sub>s. Consequently, this work does not allow evaluating the role of OR subtypes in the arrhythmogenic effects of opioids.

MR 2266 also exhibits an antiarrhythmic effect. Therefore, the combined use of MR 2266 and U50488 could eliminate the arrhythmogenic effect of U50488 regardless of OR blockade. Later it was shown that intravenous administration of dynorphin (300 nmol/kg) diminished the arrhythmogenic effect of coronary artery occlusion in rats.<sup>265</sup> This effect was abolished by pretreatment with naloxone (1 mg/kg). However, since naloxone itself exhibits an antiarrhythmic effect, it remains unclear whether the inhibition of the proarrhythmic effect of dynorphin by naloxone occurred due to the blockade of ORs or the antiarrhythmic effect of naloxone overshadowed the proarrhythmic effect of dynorphin. Interestingly, according to others, dynorphin A1-13 (25 nmol/kg) prevents the occurrence of ventricular fibrillation in anesthetized cats subjected to occlusion of the left coronary artery.<sup>266</sup> In 2003, Coles *et al.* published a comparative study of the cardiovascular effects of opioids in pigs with coronary artery occlusion (45 min) and reperfusion (3h).<sup>267</sup> They found that the preferential  $\delta$  OR agonist DADLE (1 mg/kg) and the preferential  $\kappa$  OR agonist pentazocine (5 mg/kg) aggravated the arrhythmogenic effect of coronary artery occlusion. The selective  $\kappa$  OR antagonist nor-binaltorphimine (1.5 mg/kg) exhibited the same effect. However, pretreatment with nor-binaltorphimine completely abolished the proarrhythmic effect of both DADLE and pentazocine. The authors concluded that  $\kappa$  OR activation during ischemia exhibits proarrhythmic effect in pigs.<sup>267</sup> Indeed, it is known that DADLE can activate  $\kappa$  ORs in isolated hearts and we cannot exclude the possibility that its proarrhythmic effect is mediated by activation of these receptors.<sup>143</sup> However, we would like to draw the readers' attention to the following paradox: the  $\kappa$  OR antagonist nor-binaltorphimine also had a pro-arrhythmic

effect that disappeared when treatment with the  $\kappa$  OR antagonist was combined with the  $\kappa$  OR agonist during ischemia. It is worth mentioning that the authors used high doses of the OR agonists. Meanwhile it is well known that opioid peptides at high concentration may interact with non-ORs.<sup>268-270</sup> Therefore, there is a possibility that the toxic effects of DALDA and pentazocine are unrelated to the ORs but mediated via stimulation of other receptors.

It is worth mentioning that we have never observed an arrhythmogenic or proarrhythmic effect of opioids administered intravenously to rats or in the experiments on isolated perfused rat heart. We noticed a proarrhythmic effect of DADLE only in pigs during coronary artery occlusion at a high dose of 1 mg/kg.<sup>101</sup> However, we do not exclude the possibility that opioids may have arrhythmogenic and proarrhythmic effects associated with the activation of central ORs when used at a high dose. We have established that the  $\kappa_1$  OR agonist U-50488, the  $\kappa$  OR agonist [D-Ala2]-Dynorphin A(1-13) and the preferential  $\kappa_2$  OR agonist (-)-bremazocine administered intracerebroventricularly potentiate the arrhythmogenic effect of intravenous epinephrine.<sup>271,272</sup> The effect of U-50,488 was completely blunted.<sup>271,272</sup> Pretreatment with N-cholinergic receptor antagonist hexamethonium prevented proarrhythmic effects of the intracerebroventricular administration of U50488 and dynorphin.<sup>271</sup> In contrast, intravenous administration of the preferential  $\kappa_2$  OR agonist (-)-bremazocine and intraperitoneal injection of the selective  $\kappa$  OR agonist spiradoline blunted the arrhythmogenic impact of epinephrine.<sup>272</sup> This effect was abolished by pretreatment with nor-binaltorphimine but not hexamethonium or atropine. These data indicate that stimulation of the central  $\kappa$  OR may promote a proarrhythmic effect mediated by the autonomic nervous system. Stimulation of peripheral  $\kappa$  OR may have an antiarrhythmic effect which is independent of the autonomic regulation of heart rhythm.

Thus, we do not exclude the possibility that high doses of opioids may have proarrhythmic and arrhythmogenic effects in humans and animals associated with the

activation of non-ORs or activation of central  $\kappa$  ORs. There is also a possibility that activation of a cardiac  $\kappa$  OR subtype can also contribute to the appearance of ventricular arrhythmias.

### **15. Antiarrhythmic actions of opioid antagonists**

There are reports that the OR antagonists can also exhibit antiarrhythmic properties during I/R of heart.<sup>273-277</sup> These investigations showed that intravenous administration of naloxone (1 mg/kg) before coronary artery occlusion in anaesthetized dogs reduced the incidence and severity of cardiac arrhythmias during coronary artery occlusion and reperfusion.<sup>273</sup> Studies also indicate that pretreatment with the  $\kappa_2$  OR antagonist quadazocine (3 mg/kg) or the OR antagonist (-)-Mr 1452 (4 mg/kg) prevents the appearance of arrhythmias induced by coronary artery occlusion in rats.<sup>274</sup> These investigators established that naloxone (0.5 mg/kg), the preferential  $\kappa$  OR antagonist Mr 2266 (4 mg/kg), or the non selective OR antagonist MrZ 2593 (a quarternary complex of naloxone which does not readily cross the BBB at 1 mg/kg) all prevent the appearance of arrhythmias evoked with regional cardiac ischemia in rats.<sup>275</sup> It has been reported that intravenous administration of the nonselective OR antagonist nalmephe (1 mg/kg) prevents reperfusion-induced arrhythmias in dogs.<sup>276</sup> There was also a report that pretreatment with naltrexone (2 mg/kg) or methylnaltrexone (2 mg/kg), a quaternary derivative of naltrexone that does not cross the BBB, prevents ventricular fibrillation induced with coronary artery occlusion in rabbits.<sup>277</sup>

Our studies found that the OR antagonists (naltrexone, naloxone methiodide, naltriben, BNTX, quadazocine, nor-binaltorphimine, CTAP,  $\beta$ -funaltrexamine, ICI-174,864) had no effect on the incidence of ventricular arrhythmias during coronary artery occlusion/reperfusion in rats.<sup>256,257,259</sup> It is unclear why our data contradict the data of the other researchers. It should be noted that many of the aforementioned studies used antagonists (Mr 1452, Mr 2266, nalmephe, methylnaltrexone) which are no longer used in OR studies



because they exhibit OR independent effects. All our studies were performed with rats and perhaps there were also species differences in the response to the OR antagonist. The authors who found an antiarrhythmic effect of naloxone and naltrexone were performed in investigations with dogs and rabbits.<sup>273,277</sup> The nonselective OR agonist pentazocine reportedly prevented the appearance of ventricular reperfusion arrhythmias in isolated rat hearts.<sup>231</sup>

### **16. The effect of comorbidities on the cardiovascular effects of opioids**

Pathological processes may significantly change the cardiovascular system response to exogenous opioids. For example Bolte *et al.*<sup>278</sup> found augmented negative inotropic and lusitropic response to administration of the selective  $\delta$  OR,  $\kappa$  OR agonists in the failing hamster heart. However, Kasper *et al.* could not find any difference in the negative inotropic effect of  $\kappa$  OR agonist U-50,488H on control and cardiomyopathic hamster cardiomyocytes.<sup>279</sup> The inhibitory action of the selective  $\kappa$  OR agonist U50 488H on  $\beta$ -adrenoceptor augmentation of voltage-dependent  $[Ca^{2+}]_i$  transients in the isolated cardiomyocytes appeared to be significantly reduced in spontaneously hypertensive rats.<sup>280</sup> In 2001, Pei *et al.* demonstrated that the effect of U50,488H on the  $[Ca^{2+}]_i$  transient in the isolated cardiomyocytes was significantly attenuated due to right ventricular hypertrophy induced by chronic hypoxia.<sup>281</sup> The authors established that  $\kappa$  OR signaling was impaired in the hypertrophied cardiomyocytes due to a defect in the coupling between  $\kappa$  OR and PKC. It was also found that high fat-induced obesity alters cardiovascular response to the administration of opioids in conscious rats.<sup>282</sup>

The pathological process itself may change the state of the endogenous opioid system. It has been shown that cardiomyopathy evoked an increase of the preproenkephalin A mRNA level in ventricles of hamsters.<sup>283</sup> In spontaneously hypertensive rats, the heart content of

dynorphin A was increased by 6.5-fold compared to Wistar rats.<sup>284</sup> Plasma  $\beta$ -endorphin levels are also elevated in dogs with pacing-induced congestive heart failure. Naloxone injection increased heart rate, mean aortic pressure, first derivative of left ventricular pressure and cardiac output in these dogs while in the intact animals, naloxone did not affect the hemodynamics.<sup>285</sup> These data suggest the involvement of endogenous opioids in the pathogenesis of pacing-induced congestive heart failure. This hypothesis can be supported by the data of Imai *et al.*<sup>286</sup> They found that not only naloxone but also the selective  $\delta$  OR antagonist ICI-154,129 increased mean aortic pressure, cardiac output and positive first derivative of left ventricular pressure in dogs with artificially induced right heart failure. Constriction of the aorta induced an elevation of  $\beta$ -endorphin level in blood plasma of rats.<sup>287</sup> In 1995, Oldroyd *et al.* found that plasma  $\beta$ -endorphin was increase by 29% in patients with acute heart failure and by 71% in patients with cardiogenic shock.<sup>288</sup> However, the level of this opioid in plasma was decreased in spontaneously hypertensive hamsters.<sup>389</sup> It was demonstrated that the decrease in left ventricular systolic pressure after administration of the  $\kappa$  OR agonist U50488H was attenuated in these hamsters.

These results show that pathological process can exert a significant effect on the endogenous opioid system. Can the accompanying pathological process change the cardioprotective effect of opioids? It has been demonstrated that aging does not alter a cardioprotective effect of BW373U86, a selective  $\delta$  OR agonist.<sup>168</sup> However, in 2007, Peart *et al.* reported that the selective  $\delta$  OR agonist DPDPE only improves contractile recovery after reperfusion of isolated mouse hearts from young animals.<sup>228</sup> They found that aging-related loss of  $\delta$ -opioid-mediated cardioprotection involves failure to activate p38 MAPK (mitogen activated protein kinase) and HSP27 (heat shock proteins). Gross's group demonstrated that morphine did not limit the infarct size in rats with streptozotocin-induced diabetes. This lack of protective effect was associated with the loss of coupling between ORs and glycogen

synthase kinase  $3\beta$  (GSK- $3\beta$ ).<sup>290</sup> It was also shown that remifentanil reduced myocardial infarct size and prevented apoptosis of cardiomyocytes evoked by ischemia/reperfusion in non-diabetic rats but not in rats with streptozotocin-induced diabetes.<sup>89</sup> Gross's group's data were confirmed by Chen *et al.*<sup>199</sup> They demonstrated that sufentanil reduced myocardial infarct size in the non-diabetic rats, but not those with streptozotocin-induced diabetes. The GSK- $3\beta$  inhibitor SB216763 reduced infarct size in both non-diabetic and diabetic rats. The authors concluded that absence of opioid-induced tolerance of rat heart to reperfusion injury in diabetic animals is the result of impaired interaction of ORs and GSK- $3\beta$ .<sup>199</sup>

The results of these studies cannot be mechanically applied to the humans because the most common form of diabetes in humans is a type 2 diabetes mellitus characterized by insulin resistance. Streptozotocin causes damage to the  $\beta$  cells of Langerhans islets and leads to decreased insulin secretion. Therefore streptozotocin-induced diabetes is most similar to insulin-dependent type 1 diabetes mellitus in humans. However, Tsang *et al.* showed in experiments using a rat model of type 2 diabetes mellitus (Goto-Kakizaki rats) that diabetes depresses the PI3K/Akt pathway during IP causing loss of protection. But the elevated threshold for Akt phosphorylation and protection can be reached by simply increasing the number of IP cycles.<sup>291</sup> Since this same signaling pathway is involved in the protective mechanism of opioids, as we discuss in the subsection "Signaling Mechanism of the Cardioprotective Effect of Opioids" above, it seems likely that an increasing the dose of an OR agonist may still be able to protect the diabetic heart against I/R injury. Taken together, the presented data suggest that diabetes and age may undermine the efficacy of opioid-induced cardioprotective effects. It remains unknown whether atherosclerosis, arterial and pulmonary hypertension, myocardial hypertrophy or heart failure might also alter their protection. The phenomenon of heart resistance to cardioprotective stimuli due to co-morbid diseases is potentially a serious problem in the translation of cardioprotective interventions to

clinical practice.<sup>292</sup> However, this problem may be solved by either increasing the stimulus or by direct activation of the downstream components of the signaling pathways such as p38 MAPK/HSP27 or inhibition of GSK-3 $\beta$ , which remain relatively intact in these conditions.

### **17. Prospects for the use of opioid receptor agonists in cardiological practice**

It has been found that the chronic administration of morphine increases cardiac tolerance to ischemia and reperfusion.<sup>293-297</sup> The signaling mechanism of the cardioprotective effect of chronic morphine administration differs from that of acute administration of morphine.<sup>296</sup> Chronic  $\kappa$ -OR stimulation prevents isoprenaline-induced cardiac hypertrophy and fibrosis.<sup>298</sup> Unfortunately, morphine and heroin in chronic administration cause rapid formation of drug dependence.<sup>299</sup> The formation of opioid dependence is associated with activation of central  $\mu$  OR.<sup>300</sup> The ability to form a dependence is much less pronounced in the  $\delta$  OR or  $\kappa$  OR agonists.<sup>300</sup> Therefore,  $\delta$  OR and  $\kappa$  OR agonists are not on the DEA controlled substances list. However, since the  $\kappa$  OR agonists cause dysphoria, their indication for chronic use is also unlikely.<sup>301</sup> In our opinion, the most promising agents for chronic use are peptide OR agonists, that poorly penetrate the blood-brain barrier.<sup>22,23,24</sup> These include the selective  $\mu$  OR agonist DALDA (NH<sub>2</sub>-Tyr-D-Arg-Phe-Lys-NH<sub>2</sub>)<sup>227</sup> and non-selective  $\mu$  OR and  $\delta$  OR agonist dalargin (H-Tyr-D-Ala-Gly-Phe-Leu-Arg-OH).<sup>302</sup> Our experiments have shown that dalargin exhibits antifibrillatory properties.<sup>239</sup> In Russia, this drug is used to treat stomach ulcers.<sup>303</sup> We have shown that a quaternary analogue of the  $\kappa$  opioid agonist U-50488 is another opioid that does not penetrate the BBB and is capable of increasing cardiac tolerance to ischemia/reperfusion.<sup>123</sup> Therefore, we suggest that those opioids, which are not able to activate the central ORs after systemic administration might be most amenable to chronic use as cardioprotectants in high risk cases.

Based on the data accumulated by our and Gross's group,<sup>129</sup> the most promising compounds for development of drugs, which acutely increase the heart's tolerance to the detrimental effects of ischemia and reperfusion are deltorphin II, ICI 199,441 and Eribis peptide 94. Following clinical trials, these compounds or their analogs may find use in the treatment of acute myocardial infarction or in surgical patients receiving coronary bypass.

### **18. Signaling Mechanism of the Cardioprotective Effect of Opioids**

The data described below are summarized in the signaling scheme for the cardioprotective effect of opioids presented in Figure 1. The phenomenon of ischemic preconditioning was found to be triggered by activation of  $G_{i/o}$ -coupled adenosine receptors that activate PKC and ultimately protects by inhibiting MPT early in reperfusion.<sup>304</sup> Soon it was discovered that bradykinin and OR act in parallel with the adenosine receptors as all 3 are populated during a preconditioning ischemia. Occupation of any one receptor type can put the heart into a conditioned state. It is hypothesized that ORs couple with  $G_{i/o}$  proteins which then inhibit adenylyl cyclase and activate phospholipase C which, in turn, synthesizes diacylglycerols stimulating protein kinase C.<sup>248</sup> Therefore, investigators have attempted to determine if the cardioprotective effect of opioids is associated with  $G_{i/o}$  protein activation. In a study with rats, it was shown that the  $G_{i/o}$  protein inhibitor pertussis toxin eliminated the infarct reducing effect of the  $\delta_1$  OR agonist TAN-67.<sup>91</sup> Later experiments with isolated perfused murine heart demonstrated that pertussis toxin, which rybosylates the  $\alpha_i$  subunit of the  $G_{i/o}$  protein, eliminated the cardioprotective effect of morphine.<sup>296</sup> Currently it is hypothesized that  $G_{i/o}$  proteins serve as an intermediary link between OR and the protein kinases which perform the protective signaling.

#### ***A. OR Protect Through PKC***

In 1998, a study with isolated perfused rabbit heart it was shown that the protein kinase C (PKC) inhibitor chelerythrine abolishes the infarct sparing effect of morphine.<sup>81</sup> In the following investigation performed with the isolated perfused rat heart it was found that pretreatment with chelerythrine eliminates the cardioprotective effects evoked by  $\delta$  and  $\kappa_1$  OR in vitro stimulation.<sup>95,105,173,215</sup>

In 2001, Gross's group sought to identify which PKC isoforms are involved in the infarct limiting effect of opioids.<sup>305</sup> They established that the cardioprotective effect of TAN-67 and DADLE did not occur after inhibition of all PKCs with chelerythrine and after pretreatment with the selective PKC- $\delta$  inhibitor rottlerin. They concluded that the cardioprotective effect of  $\delta$  OR agonists is mediated via PKC- $\delta$  activation. This result surprised us because PKC  $\delta$  activation and translocation to mitochondria also promotes cardiomyocyte apoptosis<sup>306</sup> and inhibition of PKC  $\delta$  enhances cardiac tolerance to reperfusion injury.<sup>307</sup> Also preconditioning's protection of adult rabbit cardiomyocytes could be blocked by a PKC  $\epsilon$ -specific antagonist but not one for PKC  $\delta$ .<sup>308</sup> However, more recent studies revealed that rottlerin is not a specific PKC- $\delta$  inhibitor.<sup>309</sup> The key role of PKC in signaling mechanism of anti-necrotic effect of opioids was confirmed in later works.<sup>110,296,310</sup>

In a 2001 study, isolated cardiomyocytes were assessed for apoptosis after exposing the cells to hypoxia (12 hr) and reoxygenation (12 hr). This study indicated that the selective  $\delta$  agonist BW373U86 prevented apoptosis of cardiomyocytes. Pretreatment with Go-6976, an inhibitor PKC- $\alpha$  and PKC- $\beta$ , abolished this protective effect of BW373U86.<sup>158</sup> This study showed the anti-apoptotic effect of the  $\delta$  OR agonist can be attributed to PKC- $\alpha$  and PKC- $\beta$  activation.

A number of studies have demonstrated that PKC is involved in the antinecrotic and antiapoptotic effects of opioids. Debate still revolves around the question of which PKC

isoforms are involved in the opioid-induced enhancement of cardiac tolerance to ischemia/reperfusion.

### ***B. PI3 kinase, Akt, and MAPK- the RISK Pathway***

Another set of kinases shown to be in the conditioning pathway are PI3 kinase, Akt and p42/p44 MAPK. These kinases are collectively called the Reperfusion Injury Survival Kinase (RISK) pathway and are downstream of PKC and are involved in mediating the protection early in reperfusion.<sup>311</sup> Isolated rabbit cardiomyocytes were subjected to hypoxia and reoxygenation and the endogenous  $\mu$  and  $\delta$  OR agonist met-enkephalin reduced cardiomyocytes death. Pretreatment with the PI3 kinase inhibitor LY-294002 abolished the cytoprotective effect.<sup>312</sup> We have found that intravenous U-50,488 decreases the IS/AAR in rats with coronary artery occlusion and the PI3 kinase inhibitor wortmannin abolished the infarct sparing effect.<sup>313</sup> PI3 Kinase phosphorylates membrane phosphoinositide at the 3 position and this product activates phosphoinositide-dependent kinases (PDKs) which activates Akt by phosphorylating it. Recently, it has been shown that the infarct limiting effect of sufentanil *in vivo* also was abolished by pretreatment with wortmannin.<sup>314</sup>

Gross's group found that intravenous administration of morphine (0.3 mg/kg) in rats induces an increase in the phosphorylation of cardiac Akt early in reperfusion.<sup>108,116</sup> The same group utilizing an isolated murine heart found that DPDPE evoked phosphorylation of Akt.<sup>228</sup> These data were confirmed by Huang *et al.*<sup>52</sup> It was also shown that the phosphorylation of Akt was involved in the cardioprotective effect of remifentanyl<sup>162</sup> and morphine.<sup>315</sup> Based on the aforementioned studies it appears that PI3 kinase and Akt are involved in cardioprotective effect of opioids.

Mitogen activated protein kinases (MAPK) comprise a family of kinases involved in growth and cytoprotection. They include p38 MAPK, JNK, p42 MAPK and p44 MAPK. The

last two isoforms are also known as Extracellular Receptor Kinase (ERK). Like all MAPKs, ERK1/2 are activated by two upstream kinase kinases, MEK1/2 which can be inhibited by PD 098059. PD 098059 completely abolishes the anti-infarct effect of TAN-67. Furthermore, TAN-67 increased the phosphorylation ERKs during reperfusion and this was prevented by PD 098059.<sup>316</sup> Ikeda and colleagues found that intravenous administration of the  $\delta$  OR agonist DADLE (1 mg/kg) increased the phosphorylation of both isoforms of ERK in the myocardium.<sup>94</sup> PD 098059 abolished both ERK's phosphorylation and the protection. Van Winkle's group published a study where isolated rabbit cardiomyocytes subjected to hypoxia and reoxygenation were protected with met-enkephaline and experienced increased phosphorylation of ERK1/2. Pretreatment with PD 098059 or the selective MEK1/2 inhibitor U-0126 abolished both effects.<sup>312</sup> The anti-infarct effect of U50488H could be blocked by an ERK inhibitor but not by one for the PI3K-Akt pathway.<sup>317</sup> Ha and colleagues also found that the non-selective OR agonist remifentanyl before reperfusion promotes an increase in the phosphorylation of ERK1/2 which could be reversed by naloxone or by a non-selective adenosine receptor inhibitor.<sup>200</sup> The A2b adenosine receptor has been proposed to be upstream of ERK in the preconditioning pathway.<sup>304</sup>

### ***C. Other protective kinases***

In 2011, a study by Li et al. indicated that morphine can ameliorate myocardial contractile dysfunction and limit infarct size following ischemia and reperfusion by a mechanism involving activation of AMPK (AMP-activated protein kinase).<sup>161</sup>

JNK and P38 MAPK are also members of the MAPK family that have been implicated in cardioprotection. Despite an increase in phosphorylation of both p38 MAPK and JNK by TAN 67, its cardioprotective effect could not be blocked by the p38 MAPK inhibitor SB-



203580.203.<sup>228,305</sup> Other groups also find an increased phosphorylation of p38 MAPK after morphine use.<sup>189,318</sup>

Morphine induces phosphorylation of GSK-3 $\beta$  and JAK2.<sup>116,290</sup> Phosphorylation of GSK-3 $\beta$  has been proposed to directly inhibit the opening of MPT and is currently regarded as a critical component in the conditioning pathway.<sup>304</sup> Phosphorylation of GSK-3 $\beta$  inhibits its kinase activity and the GSK-3 $\beta$  inhibitor SB-216763 mimicks morphine's protection. Morphine's protection is lost after inhibition of JAK2. JAK2 is a member of the SAFE pathway has been implicated in the mechanism of the heart's conditioning phenomenon.<sup>304</sup> It is unclear whether JAK directly phosphorylates GSK-3 $\beta$  kinase or acts indirectly through other kinases. DPDPE contributes to phosphorylation p70S6 kinase and GRK2 in isolated rat heart<sup>228</sup> however, it is not known whether these effects have any relevance to its protective effect.

The endothelial nitric oxide synthase (eNOS) has been implicated in the preconditioning's trigger pathway between G<sub>i/o</sub> coupled receptors and PKC.<sup>304</sup> We have found that the infarct sparing and antiarrhythmic effects of deltorphin II disappears after pretreatment with the eNOS inhibitor L-NAME.<sup>117</sup> We have shown that pretreatment with L-NAME abolished the infarct reducing effect of U-50,488.<sup>313</sup> In a study of the cardioprotective effect of Eribis peptide 94 similar data were noted by Gross et al.<sup>129</sup>

#### ***D. Tyrosine kinases***

The kinases discussed above phosphorylate serines or threonines in their target proteins. Another family of kinases only phosphorylate tyrosine residues. Tyrosine kinases are proposed to be involved in the transactivation of the epidermal growth factor (EGF) receptor, a step in the OR's trigger pathway for preconditioning.<sup>304</sup> A 2001 study indicated that the nonselective tyrosine kinase genistein completely abolished the infarct sparing effect

of TAN-67 and DADLE but lavendustin, an inhibitor of Src kinase and the EGF receptor, did not affect the cardioprotective effect of TAN-67 or DADLE.<sup>305</sup> These investigators concluded that neither Src kinase nor the EGF receptor are involved in the cardioprotective effect of  $\delta$  agonists but that some tyrosine kinase is involved. Quite the opposite findings were obtained by Cao and colleagues.<sup>312</sup> They subjected isolated cardiomyocytes to hypoxia and reoxygenation and the Src kinase inhibitor herbimycin A completely abolished the cytoprotective effect of met-enkephalin. Finally, we have also shown that the infarct limiting effect of deltorphin II is maintained after pretreatment with genistein.<sup>117</sup>

Studies have shown that the infarct sparing effect of morphine and  $\delta$  agonist FIT disappeared after pretreatment with AG-490, an inhibitor of the tyrosine kinase JAK2, but not with the JAK3 inhibitor ZM-449829.<sup>116</sup> Morphine induced phosphorylation of JAK2 in the area at risk.<sup>116,290</sup> It is possible that JAK2 may be the tyrosine kinase which is involved in opioid induced enhancement of cardiac tolerance to ischemia/reperfusion.

#### ***E. Interactions between adenosine receptors and ORs***

Isolated perfused rat hearts were subjected to ischemia/reperfusion and fentanyl improved the post ischemic recovery. Naloxone abolished the protection from fentanyl as did the selective adenosine A<sub>1</sub> receptor antagonist DPCPX.<sup>214</sup> These authors concluded that the protective effect of fentanyl involved both ORs and the A<sub>1</sub> receptor. Similar data were obtained by Peart and Gross, they administered either morphine or the selective adenosine A<sub>1</sub> receptor agonist CCPA to rats to rats subjected to coronary artery occlusion and either compound evoked a decrease in the IS/AAR. The infarct limiting effect of morphine was eliminated by DPCPX and after selective blocking  $\delta_1$  OR with BNTX. Furthermore, the cardioprotective effect of CCPA was blocked after injection of either DPCPX or BNTX.<sup>320</sup> Coadministration of morphine and CCPA did not offer any additive protection. These authors

concluded that there is an interaction between  $\delta_1$  OR and  $A_1$  receptor at the intracellular signaling level (cross-talk).

#### ***F. Transactivation of the EGF receptor***

In 2005, Gross's group published data on the cardioprotective effect of endogenous adenosine.<sup>321</sup> The level of adenosine was elevated with intravenous administration the adenosine kinase inhibitor 5-iodotubercidin (1 mg/kg). This compound decreased the IS/AAR 2 fold. Pretreatment with the adenosine  $A_1$  receptor-selective antagonist DPCPX, the adenosine  $A_3$  receptor-selective antagonist MRS-1523 or the  $\delta_1$  OR-selective antagonist BNTX abolished the infarct reducing effect of 5-iodotubercidin.<sup>321</sup> These data led these researchers to conclude that the cardioprotective effect of endogenous adenosine is mediated via simultaneous  $A_1$  receptor,  $A_3$  receptor and  $\delta_1$  OR activation. Recently, Ha et al. found that the non-selective OR agonist remifentanyl started 5 min before reperfusion in the isolated rat heart decreases the IS/AAR and evokes the phosphorylation of ERK1/2. These effects were blocked by pretreatment with naloxone or the non-selective adenosine receptor antagonist 8-(p-sulphophenyl) theophylline.<sup>200</sup> It is our opinion that the aforementioned studies provide evidence of interaction of adenosine and OR at some level. It has been proposed that transactivation may be involved as both receptors converge through transactivation of the EGF receptor.<sup>322,323</sup> However, it is not clear how inhibition of one could turn off signaling of the other through this transactivation.

EGF receptor activation protects against ischemia/reperfusion.<sup>312,324,325</sup> Met-enkephalin reduces cell death in isolated cardiomyocytes subjected hypoxia and reoxygenation and phosphorylates the EGF receptor. The EGF tyrosine kinase inhibitor AG-1478, the Src kinase inhibitor herbimycin A or naloxone eliminated phosphorylation of EGF receptor and the cytoprotective effect.<sup>312</sup>

The aforementioned data indicate that there is an important role of transactivation of opioid and adenosine receptors in cardiac tolerance to impact of ischemia and reperfusion. Opioid transactivation of EGF receptor tyrosine kinase is a binder link between ORs and ERK1/2 and the downstream signaling pathways including ERK and PI3 kinase. The opinion EGF receptor transactivation is an important mechanism in implementing the protective effect of opioids is now shared by other physiologists.<sup>322,323</sup>

### ***G. K<sub>ATP</sub> and BK channels***

Pretreatment with the K<sub>ATP</sub> channel blocker glibenclamide abolished anti-infarct effect of morphine.<sup>10</sup> Two years later the same group demonstrated that glibenclamide abolished the infarct reducing effect of TAN-67.<sup>91</sup> It was also shown that the cardioprotective effect of DADLE *in vitro* disappeared after K<sub>ATP</sub> channel blockade with this inhibitor.<sup>92</sup> Further studies were designed to determine what types of K<sub>ATP</sub> channels are involved in cardioprotective effect of opioids. It was observed that incubation of cardiomyocytes with morphine prevents cell death and the selective inhibitor of the mitochondrial K<sub>ATP</sub> channel (mitoK<sub>ATP</sub>) 5-hydroxydecanoate (5-HD) completely abolished the cytoprotective effect of morphine.<sup>82</sup> Pretreatment with fentanyl improved post-ischemic recovery of function and that was also dependent on the opening of mitoK<sub>ATP</sub>.<sup>214</sup> In 2000, it was found the antiarrhythmic and infarct sparing effect of TAN-67 *in vivo* was abolished by blocking mitoK<sub>ATP</sub> with 5-HD but not after blocking sarcolemmal K<sub>ATP</sub> channels (sarcK<sub>ATP</sub>) with HMR 1098.<sup>102</sup> The cytoprotective effect of TAN-67 in isolated cardiomyocytes disappeared after blocking mitoK<sub>ATP</sub> with 5-HD.<sup>105</sup> 5-HD also blocked the cardioprotective effect of U-50,488 in isolated perfused rat heart,<sup>326</sup> abolished the cytoprotective effect of morphine and  $\delta$  agonist BW373U86 in isolated chicken cardiomyocytes.<sup>107</sup> In an *in vivo* study we found that the infarct reducing effect of deltorphin II did not occur after blockade of mitoK<sub>ATP</sub> with 5-HD.<sup>117</sup> A large number of

similar publications now confirm the key role of mitoK<sub>ATP</sub> in the cardioprotective effect of opioids.

A few studies also show a role of sarcK<sub>ATP</sub> in opioid-induced cardioprotection. The cytoprotective effect of met-enkephalin disappeared after selective blockade of mitoK<sub>ATP</sub> with 5-HD or after selective blockade of sarcK<sub>ATP</sub> with HMR 1098.<sup>98,99</sup> The anti-infarct effect of morphine and BW373U86 *in vivo* was no longer observed after either mitoK<sub>ATP</sub> or sarcK<sub>ATP</sub> blockade.<sup>108</sup> In another study it was demonstrated the cardioprotective effect of Eribis peptide 94 was dependant on both mitoK<sub>ATP</sub> and sarcK<sub>ATP</sub> opening.<sup>129</sup>

In 2005, it was proposed that the mitochondrial Ca<sup>2+</sup>-dependent big conductance K<sup>+</sup> channel (mitoBK<sub>Ca</sub> channel) was involved in the cardioprotective mechanism of opioids. The κ<sub>1</sub> OR agonist U-50,488 provided a decrease in the IS/AAR in isolated rat heart and prevented cell death isolated myocytes subjected to simulated ischemia/reperfusion. The mitoBK<sub>Ca</sub> channel inhibitor paxilline abolished both effects of U-50488.<sup>327</sup>

#### ***H. Redox signaling***

The above studies indicate that mitoK<sub>ATP</sub> and perhaps sarcK<sub>ATP</sub> are involved in the cardioprotective effect of opioids. It was originally thought that K<sub>ATP</sub> must be end effectors of the protection, however, evidence now indicates that their role is primarily one of signal transduction. Studies with preconditioning provided strong evidence that opening of mitoK<sub>ATP</sub> activate PKC through redox signaling with free radicals<sup>304</sup> and that seems to include signaling from the ORs. The anti-infarct effect of morphine in rabbit heart could be blocked with the free radical scavenger N-2-mercaptopropionyl glycine (MPG).<sup>328</sup> 10 min preconditioning with morphine or BW373U86 increased cell survival of isolated chicken cardiomyocytes subjected to hypoxia and reoxygenation. Morphine-induced protection and free radical production was abolished by MPG, naloxone, BNTX, a selective δ<sub>1</sub> OR antagonist; or 5-HD. The superoxide dismutase inhibitor diethyldithiocarbamic acid exhibited the same effect. Finally the increase

in oxygen radicals was abolished by the mitochondrial electron transport inhibitor myxothiazol.<sup>329</sup> It was also shown later that the infarct sparing effect of morphine does not occur after blocking ROS production with MPG.<sup>320</sup> In a study with isolated cardiomyocytes subjected to hypoxia/reoxygenation, it was shown that the cytoprotective effect of morphine disappeared after pretreatment with MPG.<sup>315</sup>

Thus, the above data indicate that opioid induced opening of mitoK<sub>ATP</sub> leads to increased production of ROS which through redox signaling enhances of cardiac tolerance to ischemia/reperfusion.

### ***I. MPT***

Elevated cytosolic ROS and Ca<sup>2+</sup> in the first minutes of reperfusion are thought to open MPT (mitochondrial permeability transition pores).<sup>156</sup> MPT can destroy the mitochondria and either kill the cardiomyocyte outright (necrosis) or release proapoptotic substances depending on how many mitochondria are lost. A 2005 study indicated that MPT are involved in the cardioprotective effect of opioids.<sup>327</sup> The regional ischemia and reperfusion was carried out in the isolated perfused rat heart. U-50488 decrease in the IS/AAR and lactate dehydrogenase in the coronary effluent of isolated hearts. The MPT opener atractyloside abolished the cardioprotective effect of U-50488.<sup>327</sup> It is assumed that OR stimulation inhibited MPT opening at reperfusion and opening MPT directly with atractyloside overrode any inhibition from protective signaling. Mitochondria isolated from the ischemic zone of rat hearts receiving morphine have an elevated threshold for opening MPT with Ca<sup>2+</sup> and this resistance to MPT opening was lost if PI3 kinase was inhibited with wortmannin.<sup>330</sup> In our *in vivo* study we demonstrated that U-50,488 induces an infarct reducing effect as well as an antiapoptotic effect. It also decreases the activity of caspases which become activated after MPT opening.<sup>163</sup> The infarct sparing and antiapoptotic effects disappeared after blockade of mitoK<sub>ATP</sub> with 5-HD. U-50,488 evoked an enhancement of

expression of antiapoptotic protein Bcl-2 and decreased the expression of proapoptotic protein Bax.<sup>163</sup> Presumably, the  $\kappa_1$  OR agonist prevented MPT opening with signaling through mitoK<sub>ATP</sub> as discussed above. Kim et al. also found that remifentanyl not only limits infarction but also increased Bcl-2 and decreased Bax.<sup>89</sup> We conclude that the current evidence indicates that the end effector of the cardioprotective effect of opioids is inhibition of the MPT early in reperfusion.

### **19. Will an OR agonist provide protection to today's patient population?**

The ORs appear to protect through a signaling pathway similar if not identical to that of IP and IPost. Evidence suggests that the loading doses of P2Y<sub>12</sub> receptor inhibitors that are now routinely given to all patients undergoing reperfusion therapy to prevent platelet aggregation in their stents may also trigger this same protection could make the OR agonist redundant. The P2Y<sub>12</sub> blocker cangrelor limited infarct size when present at reperfusion in open-chest rabbits by an amount similar to that with IPost. Protection from cangrelor could be blocked by 5-HD, wortmannin, adenosine receptor inhibitors, an ERK inhibitor, or the antioxidant N-2-mercaptopropionyl glycine. These same signaling inhibitors will block protection from both IP and IPost. None of those agents restored the ability of platelets to aggregate indicating that protective signaling rather than prevention of thrombi was responsible for the protection. When cangrelor and IPost were combined in rabbits, IPost caused no additional protection, probably because both protect by the same mechanism.<sup>331</sup>

Recent attempts to translate IPost to clinical practice in the setting of acute myocardial infarction have been disappointing. The first IPost trial was performed just before P2Y<sub>12</sub> blockers came into widespread use and was very positive but all of those performed thereafter had P2Y<sub>12</sub> blockers present in all patients and showed minimal or no protection from adding IPost.<sup>332</sup> These data indicate that all of today's patients receiving primary angioplasty to

reperfuse their coronary arteries are already in a postconditioned state from their P2Y<sub>12</sub> blocker loading dose. These drugs were quickly added to the guidelines because they greatly improved clinical outcomes. Their benefit was assumed to result from preventing intracoronary thrombi and the possibility of a direct anti-infarct effect was not considered. Of course today it would be impossible to do a clinical trial to measure their ability to reduce infarct size in man because it would be unethical to deny platelet inhibitors to a control group. Any clinically effective agent today must, therefore, be able to provide additional protection when combined with a P2Y<sub>12</sub> inhibitor. To date no OR agonists have been tested to see if any of them can provide additional protection in an animal model treated with a P2Y<sub>12</sub> inhibitor. That screening should be done before considering a large scale clinical trial of any cardioprotectant.

## 20. Concluding Remarks

Stimulation of central  $\mu$  OR promotes reduction of infarct size during coronary artery occlusion and reperfusion. Occupancy of peripheral  $\mu$  OR by opioids promotes better recovery of cardiac contractile function after ischemia. Activation of peripheral and possibly central  $\delta_1$  ORs prevents cardiomyocyte necrosis, apoptosis and arrhythmias caused by ischemia/reperfusion of the heart. Stimulation of peripheral OR  $\delta_2$  reduces infarct size and inhibits arrhythmogenesis during coronary artery occlusion and reperfusion. Activation of peripheral  $\kappa_1$  ORs also prevents cardiomyocyte necrosis, apoptosis and arrhythmias induced by ischemia/reperfusion. Stimulation of peripheral  $\delta$  and  $\kappa$  ORs contributes to increase in cardiac contractility during reperfusion. No data are available on the involvement of ORL1 receptor in the increased cardiac tolerance to ischemia/reperfusion by opioids. It should be noted however, that at high dose opioids may contribute to cardiac arrhythmias and this effect is probably mediated by non-opioid receptor activation.



The data show that the  $\mu$ ,  $\delta 1$ ,  $\delta 2$  and  $\kappa 1$  OR agonists all are promising candidates for a drug, which would enhance the cardiac tolerance to I/R. The OR agonists exert infarct-reducing effects both with prophylactic administration and with acute treatment just prior to reperfusion. Furthermore, opioids are also effective in preventing ischemia-induced arrhythmias.

### **Declaration of Conflicting Interests**

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

### **Acknowledgements**

The authors would like to thank Professor Saadeh Suleiman and Professor Raimondo Ascione from the University of Bristol (UK) for useful advice.

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Professor Leonid N. Maslov and Dr. Natalia Naryzhnaia are currently supported by a grant from the Russian National Foundation (grant 14-15-00008). Dr. Igor Khaliulin is currently supported by a grant from the British Heart Foundation (grant No. PG/15/66/31710).

## References

1. Girn HR, Ahilathirunayagam S, Mavor AI, Homer-Vanniasinkam S. Reperfusion syndrome: cellular mechanisms of microvascular dysfunction and potential therapeutic strategies. *Vasc Endovascular Surg* 2007;41:277-293.
2. Monassier JP. Reperfusion injury in acute myocardial infarction. From bench to cath lab. Part I: Basic considerations. *Arch Cardiovasc Dis* 2008;101:491-500.
3. Ostadal B, Kolar F. *Cardiac Ischemia: From Injury to Protection*. Boston, Dordrecht, London: Kluwer Academic Publishers, 1999. 173 p.
4. Sharma V, Bell RM, Yellon DM. Targeting reperfusion injury in acute myocardial infarction: a review of reperfusion injury pharmacotherapy. *Expert Opin Pharmacother* 2012;13:1153-1175.
5. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124-1136.
6. Yellon DM, Baxter GF. A "second window of protection" or delayed preconditioning phenomenon: future horizons for myocardial protection? *J Mol Cell Cardiol* 1995;27:1023-1034.
7. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2003;285:H579-H588.
8. Liu GS, Thornton J, Van Winkle DM, Stanley AW, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. *Circulation* 1991;84:350-356.
9. Schultz JE, Rose E, Yao Z, Gross GJ. Evidence for involvement of opioid receptors in ischemic preconditioning in rat hearts. *Am J Physiol* 1995;268:H2157-H2161.
10. Schultz JE, Hsu AK, Gross GJ. Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart. *Circ Res* 1996;78:1100-1104.
11. Kin H, Zatta AJ, Jiang R, Reeves JG, Mykytenko J, Sorescu G, Zhao ZQ, Wang NP, Guyton RA, Vinten-Johansen J. Activation of opioid receptors mediates the infarct size reduction by postconditioning. *J Mol Cell Cardiol* 2005;38:827.
12. Marson L, Kiritsy-Roy JA, Van Loon GR.  $\mu$ -Opioid peptide modulation of cardiovascular and sympathoadrenal responses to stress. *Am J Physiol* 1989;257:R901-R908.
13. Panksepp J, Bishop P. An autoradiographic map of ( $^3$ H)diprenorphine binding in rat brain: effects of social interaction. *Brain Res Bull* 1981;7:405-410.

14. Bunzow JR, Saez C, Mortrud M, Bouvier C, William JT, Low M, Grandy DK. Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a mu, delta, or kappa opioid receptor type. *FEBS Lett.* 1994;347:284-288.
15. Borghi V, Przewlocka B, Labuz D, Maj M, Ilona O, Pavone F. Formalin-induced pain and  $\mu$ -opioid receptor density in brain and spinal cord are modulated by A1 and A2a adenosine agonists in mice. *Brain Res* 2002;956:339-348.
16. Bailey A, Ledent C, Kelly M, Hourani SM, Kitchen I. Changes in spinal  $\delta$  and  $\kappa$  opioid systems in mice deficient in the A2A receptor gene. *J. Neurosci.* 2002;22:9210-9220.
17. Bridge KE, Wainwright A, Reilly K, Oliver KR. Autoradiographic localization of  $^{125}\text{I}$  [Tyr $^{14}$ ] nociceptin/orphanin FQ binding sites in macaque primate CNS. *Neuroscience* 2003;118:513-523.
18. Pradhan AA, Clarke PB. Comparison between  $\delta$ -opioid receptor functional response and autoradiographic labeling in rat brain and spinal cord. *J Comp Neurol* 2005;481:416-426.
19. Diaz SL, Barros VG, Antonelli MC, Rubio MC, Balerio GN. Morphine withdrawal syndrome and its prevention with baclofen: Autoradiographic study of mu-opioid receptors in prepubertal male and female mice. *Synapse* 2006;60:132-140.
20. Homberg JR, Mul JD, de Wit E, Cuppen E. Complete knockout of the nociceptin/orphanin FQ receptor in the rat does not induce compensatory changes in  $\mu$ ,  $\delta$  and  $\kappa$  opioid receptors. *Neuroscience* 2009;163:308-315.
21. Lalanne L, Ayranci G, Kieffer BL, Lutz PE. The kappa opioid receptor: from addiction to depression, and back. *Front Psychiatry* 2014;5:170.
22. Cornford EM, Braun LD, Crane PD, Oldendorf WH. Blood-brain barrier restriction of peptides and the low uptake of enkephalins. *Endocrinology* 1978;103:1297-1303.
23. Samii A, Bickel U, Stroth U, Pardridge WM. Blood-brain barrier transport of neuropeptides: analysis with a metabolically stable dermorphin analogue. *Am J Physiol* 1994;267:E124-E131.
24. Lishmanov IuB, Maslov LN, Rice K. Blood-brain barrier permeability for the ligands of opioid receptors. *Eksp Klin Farmakol.* 2002;65:71-77. (in Russian)
25. Freye E, Hartung E, Schenk GK. Perfusion of the fourth cerebral ventricle with the synthetic opioid peptide, FK 33-824, induces dose-related bradycardia and naloxone-reversible respiratory depression in the awake dog. *Pharmacology* 1982;25(1):6-11.
26. Inoue K, Nashan B, Arndt JO. [D-Met $^2$ ,Pro $^5$ ]enkephalinamide activates cardioinhibitory efferents in anaesthetized dogs. *Eur J Pharmacol* 1985;110:233-239
27. Haddad GG, Jeng HJ, Lai TL. Effect of endorphins on heart rate and blood pressure in adult dogs. *Am J Physiol* 1986;250:H796-H805.

28. Kiritsy-Roy JA, Marson L, Van Loon GR. Sympathoadrenal, cardiovascular and blood gas responses to highly selective mu and delta opioid peptides. *J Pharmacol Exp Ther* 1989;251:1096-1103.
29. Wittert G, Hope P, Pyle D. Tissue distribution of opioid receptor gene expression in the rat. *Biochem Biophys Res Commun* 1996;218:877-881.
30. Krazinski BE, Koziorowski M, Brzuzan P, Okrasa S. The expression of genes encoding opioid precursors and the influence of opioid receptor agonists on steroidogenesis in porcine adrenocortical cells in vitro. *J Physiol Pharmacol* 2011;62:461-468.
31. Wang HB, Guan JS, Bao L, Zhang X. Distinct subcellular distribution of  $\delta$ -opioid receptor fused with various tags in PC12 cells. *Neurochem Res* 2008;33:2028-2034.
32. Burnie J. Naloxone in shock. *Lancet* 1981; 1: 942.
33. Dumont M, Lemaire S. Increased content of immunoreactive Leu-enkephalin and alteration of  $\delta$  opioid receptor in hearts of spontaneously hypertensive rats. *Neurosci Lett* 1988;94:114-118.
34. Ventura C, Bastagli L, Bernardi P, Caldara CM, Guarnieri C. Opioid receptors in rat cardiac sarcolemma: effect of phenylephrine and isoproterenol. *Biochim Biophys Acta* 1989;987:69-74.
35. Tai KK, Jin WQ, Chan TK, Wong TM. Characterization of [ $^3$ H]U69593 binding sites in the rat heart by receptor binding assays. *J Mol Cell Cardiol* 1991;23:1297-1302.
36. Jin WQ, Tai KK, Chan TK, Wong TM. Further characterization of [ $^3$ H]U69593 binding sites in the rat heart. *J Mol Cell Cardiol* 1995;27:1507-1511.
37. Zimlichman R, Gefel D, Eliahou H, Matas Z, Rosen B, Gass S, Ela C, Eilam Y, Vogel Z, Barg J. Expression of opioid receptors during heart ontogeny in normotensive and hypertensive rats. *Circulation* 1996;93:1020-1025.
38. Ela C, Barg J, Vogel Z, Hasin Y, Eilam Y. Distinct components of morphine effects on cardiac myocytes are mediated by the  $\kappa$  and  $\delta$  opioid receptors. *J Mol Cell Cardiol* 1997; 29:711-720.
39. Weil J, Zolk O, Griepentrog J, Wenzel U, Zimmermann WH, Eschenhagen T. Alterations of the preproenkephalin system in cardiac hypertrophy and its role in atrioventricular conduction. *Cardiovasc Res* 2006;69:412-422.
40. Stefano GB, Hartman A, Bilfinger TV, Magazine HI, Liu Y, Casares F, Goligorsky MS. Presence of the  $\mu_3$  opiate receptor in endothelial cells. Coupling to nitric oxide production and vasodilation. *J Biol Chem* 1995;270:30290-30293.
41. Stefano GB, Salzet M, Hughes TK, Bilfinger TV.  $\delta_2$  opioid receptor subtype on human vascular endothelium uncouples morphine stimulated nitric oxide release. *Int J Cardiol* 1998;64:S43-S51.

42. Saeed RW, Stefano GB, Murga JD, Short TW, Qi F, Bilfinger TV, Magazine HI. Expression of functional delta opioid receptors in vascular smooth muscle. *Int J Mol Med* 2000;6:673-677.
43. Dumont M, Lemaire S. Characterization of the high affinity [<sup>3</sup>H]nociceptin binding site in membrane preparations of rat heart: correlations with the non-opioid dynorphin binding site. *J Mol Cell Cardiol* 1998;30:2751-2760.
44. Kim KW, Chung YJ, Han JH, Woo RS, Park EY, Seul KH, Kim SZ, Cho KW, Kim SH. Nociceptin/orphanin FQ increases ANP secretion in neonatal cardiac myocytes. *Life Sci* 2002;70:1065-1074.
45. Head BP, Patel HH, Roth DM, Lai NC, Niesman IR, Farquhar MG, Insel PA. G-protein-coupled receptor signaling components localize in both sarcolemmal and intracellular caveolin-3-associated microdomains in adult cardiac myocytes. *J Biol Chem* 2005;280:31036-31044.
46. McDonald J, Leonard AD, Serrano-Gomez A, Young SP, Swanevelder J, Thompson JP, Lambert DG. Assessment of nociceptin/orphanin FQ and  $\mu$ -opioid receptor mRNA in the human right atrium. *Br J Anaesth* 2010;104:698-704.
47. Sobanski P, Krajnik M, Shaqura M, Bloch-Boguslawska E, Schafer M, Mousa SA. The presence of  $\mu$ -,  $\delta$ -, and  $\kappa$ -opioid receptors in human heart tissue. *Heart Vessels* 2014; 2014; 29(6): 855-863.
48. Mousa SA, Shaqura M, Schper J, Huang W, Treskatsch S, Habazettl H, Abdul-Khaliq H, Schfer M. Identification of  $\mu$ - and  $\kappa$ -opioid receptors as potential targets to regulate parasympathetic, sympathetic, and sensory neurons within rat intracardiac ganglia. *J Comp Neurol* 2010;518:3836-3847.
49. Mousa SA, Shaqura M, Schaper J, Treskatsch S, Habazettl H, Schafer M, Abdul-Khaliq H. Developmental expression of  $\delta$ -opioid receptors during maturation of the parasympathetic, sympathetic, and sensory innervations of the neonatal heart: early targets for opioid regulation of autonomic control. *J Comp Neurol* 2011; 519:957-971.
50. Huang MH, Friend DS, Sunday ME, Singh K, Haley K, Austen KF, Kelly RA, Smith TW. An intrinsic adrenergic system in mammalian heart. *J Clin Invest* 1996;98:1298-1303.
51. Huang MH, Wang HQ, Roeske WR, Birnbaum Y, Wu Y, Yang NP, Lin Y, Ye Y, McAdoo DJ, Hughes MG, Lick SD, Boor PJ, Lui CY, Uretsky BF. Mediating  $\delta$ -opioid-initiated heart protection via the  $\beta_2$ -adrenergic receptor: role of the intrinsic cardiac adrenergic cell. *Am J Physiol Heart Circ Physiol* 2007;293:H376-H384.
52. Huang MH, Nguyen V, Wu Y, Rastogi S, Lui CY, Birnbaum Y, Wang HQ, Ware DL, Chauhan M, Garg N, Poh KK, Ye L, Omar AR, Tan HC, Uretsky BF, Fujise K. Reducing ischaemia/reperfusion injury through  $\delta$ -opioid-regulated intrinsic cardiac adrenergic cells: adreno-peptidergic co-signalling. *Cardiovasc Res* 2009;84:452-460.

53. Kett A, Omoniyi AT, Kim H, Olariu N, Wu D, Szeto HH, Clapp JF. Baroreflex-mediated bradycardia but not tachycardia is blunted peripherally by intravenous mu-opioid agonists. *Am J Obstet Gynecol* 1998;178:950-955.
54. Omoniyi AT, Wu D, Soong Y, Szeto HH. Baroreflex-mediated bradycardia is blunted by intravenous  $\mu$ - but not  $\kappa$ -opioid agonists. *J Cardiovasc Pharmacol* 1998;31:954-959.
55. Urthaler F, Isobe JH, Gilmour KE, James TN. Morphine and autonomic control of the sinus node. *Chest* 1973;64:203-212. Weihe E, McKnight AT, Corbett AD, Hartschuh W, Reinecke M, Kosterlitz HW. Characterizations of opioid peptides in guinea-pig heart and skin. *Life Sci.* 1983;33:711-714.
56. Gautret B, Schmitt H. Cardiac slowing induced by peripheral  $\kappa$ -opiate receptor stimulation in rats. *Eur J Pharmacol* 1984;102:159-163.
57. Weitzell R, Illes P, Starke K. Inhibition via opioid  $\mu$ - and  $\delta$ -receptors of vagal transmission in rabbit isolated heart. *Naunyn-Schmiedeberg's Arch Pharmacol* 1984;328:186-190.
58. Musha T, Satoh E, Koyanagawa H, Kimura T, Satoh S. Effects of opioid agonists on sympathetic and parasympathetic transmission to the dog heart. *J Pharmacol Exp Ther* 1989;250:1087-1091.
59. Malinowska B, Piszcz J, Koneczny B, Hryniewicz A, Schlicker E. Modulation of the cardiac autonomic transmission of the pithed rats by presynaptic opioid OP4 and cannabinoid CB1 receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 2001;364:233-241.
60. Jackson KE, Farias M, Stanfill AS, Caffrey JL. Transient arterial occlusion raises enkephalin in the canine sinoatrial node and improves vagal bradycardia. *Auton Neurosci* 2001;94:84-92.
61. Farias M, Jackson KE, Yoshishige D, Caffrey JL. Cardiac enkephalins interrupt vagal bradycardia via  $\delta_2$ -opioid receptors in sinoatrial node. *Am J Physiol Heart Circ Physiol* 2003;284:H1693-H1701.
62. Deo SH, Johnson-Davis S, Barlow MA, Yoshishige D, Caffrey JL. Repeated  $\delta_1$ -opioid receptor stimulation reduces  $\delta_2$ -opioid receptor responses in the SA node. *Am J Physiol Heart Circ Physiol.* 2006;291:H2246-H2254.
63. Deo SH, Barlow MA, Gonzalez L, Yoshishige D, Caffrey JL. Cholinergic location of  $\delta$ -opioid receptors in canine atria and SA node. *Am J Physiol Heart Circ Physiol* 2008;294:H829-H838.
64. Ledda F, Mantelli L. Possible presynaptic inhibitory effect of etorphine on synaptic nerve terminals of guinea-pig heart. *Eur J Pharmacol* 1982;85:247-250.
65. Ledda F, Mantelli L, Corti V, Fantozzi R. Inhibition of cardiac response to sympathetic nerve stimulation by opioid peptides and its potentiation by morphine and methadone. *Eur J Pharmacol* 1984;102:443-450.

66. Ledda F, Mantelli L, Corti V. Sensitivity to dynorphin-(1-13) of the presynaptic inhibitory opiate receptors of the guinea-pig heart. *Eur J Pharmacol* 1985;117:377-380.
67. Starke K, Schoffel E, Illes P. The sympathetic axons innervating the sinus node of the rabbit possess presynaptic opioid  $\kappa$ - but not  $\mu$ - or  $\delta$ -receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 1985;329:206-209.
68. Fuder H, Buder M, Riers HD, Rothacher G. On the opioid receptor subtype inhibiting the evoked release of  $^3\text{H}$ -noradrenaline from guinea-pig atria in vitro. *Naunyn-Schmiedeberg's Arch Pharmacol* 1986;332:148-155.
69. Hung CF, Chang WL, Liang HC, Su MJ. Identification of opioid receptors in the sympathetic and parasympathetic nerves of guinea-pig atria. *Fundam Clin Pharmacol* 2000;14:387-394.
70. Ensinger H, Hedler L, Schurr C, Starke K. Ethylketocyclazocine decreases noradrenaline release and blood pressure in the rabbit at a peripheral opioid receptor. *Naunyn Schmiedeberg's Arch Pharmacol*. 1984;328:20-3.
71. Ensinger H, Hedler L, Szabo B, Starke K. Bremazocine causes sympatho-inhibition and hypotension in rabbits by activating peripheral kappa-receptors. *J Cardiovasc Pharmacol* 1986;8:470-475.
72. Gu H, Barron BA, Gaugl JF, Caffrey JL. Dynorphin, naloxone, and overflow of norepinephrine during cardiac nerve stimulation in dogs. *Am J Physiol* 1992;263:H153-H161.
73. Feuerstein G, Zukowska-Grojec Z. Effect of dermorphin and morphine on the sympathetic and cardiovascular system of the pithed rat. *Neuropeptides* 1987;9:139-150.
74. Arndt ML, Wu D, Soong Y, Szeto HH. Nociceptin/orphanin FQ increases blood pressure and heart rate via sympathetic activation in sheep. *Peptides* 1999;20:465-470.
75. Sander GE, Given MB, Lowe RF, Wolf RH, Brizzee KR, Giles TD. The effect of area postrema lesions on hemodynamic responses to systemic methionine-enkephalin in conscious dogs. *Am J Hypertens* 1988;1:1S-3S.
76. Sander GE, Lowe RF, Given MB, Giles TD. Interactions between circulating peptides and the central nervous system in hemodynamic regulation. *Am J Cardiol* 1989;64:44C-50C.
77. Giuliani S, Maggi CA, Meli A. Opioid receptors and prejunctional modulation of capsaicin-sensitive sensory nerves in guinea-pig left atrium. *Gen Pharmacol* 1990;21:417-421.
78. Giuliani S, Maggi CA. Prejunctional modulation by nociceptin of nerve-mediated inotropic responses in guinea-pig left atrium. *Eur J Pharmacol* 1997;332:231-236.
79. Gulati A, Bhargava HN. Cardiovascular responses to  $\kappa$  opioid agonists in intact and adrenal demedullated rats. *Eur J Pharmacol* 1988;156:247-257.

80. Schultz JE, Hsu AK, Nagase H, Gross GJ. Ischemic preconditioning and morphine-induced cardioprotection involve the delta ( $\delta$ )-opioid receptor in the intact rat heart. *J Mol Cell Cardiol* 1997;29:2187-2195.
81. Miki T, Cohen MV, Downey JM. Opioid receptors contributes to ischemic preconditioning through protein kinase C activation in rabbits. *Mol Cell Biochem* 1998;186:3-12.
82. Liang BT, Gross GJ. Direct preconditioning of cardiac myocytes via opioid receptors and  $K_{ATP}$  channels. *Circ Res* 1999;84:1396-1400.
83. Wu S, Wong MC, Chen M, Cho CH, Wong TM. Role of opioid receptors in cardioprotection of cold-restraint stress and morphine. *J Biomed Sci* 2004;11:726-731.
84. Lu Y, Dong C, Yu J, Li L. Role of central and peripheral opioid receptors in the cardioprotection of intravenous morphine preconditioning. *Ir J Med Sci* 2011;180:881-885.
85. Bilir A, Erkasap N, Koken T, Gulec S, Kaygisiz Z, Tanriverdi B, Kurt I. Effects of tramadol on myocardial ischemia-reperfusion injury. *Scand Cardiovasc. J* 2007;41:242-247.
86. Wagner R, Piler P, Bedanova H, Adamek P, Grodecka L, Freiburger T. Myocardial injury is decreased by late remote ischaemic preconditioning and aggravated by tramadol in patients undergoing cardiac surgery: a randomised controlled trial. *Interact. Cardiovasc Thorac Surg*. 2010;11:758-762.
87. Zhang Y, Irwin MG, Wong TM. Remifentanyl preconditioning protects against ischemic injury in the intact rat heart. *Anesthesiology*. 2004;101:918-923.
88. Zhang Y, Irwin MG, Wong TM, Chen M, Cao CM. Remifentanyl preconditioning confers cardioprotection via cardiac kappa- and delta-opioid receptors. *Anesthesiology* 2005; 102:371-388.
89. Kim HS, Cho JE, Hwang KC, Shim YH, Lee JH, Kwak YL. Diabetes mellitus mitigates cardioprotective effects of remifentanyl preconditioning in ischemia-reperfused rat heart in association with anti-apoptotic pathways of survival. *Eur J Pharmacol* 2010;628:132-139.
90. Wong GT, Huang Z, Ji S, Irwin MG. Remifentanyl reduces the release of biochemical markers of myocardial damage after coronary artery bypass surgery: a randomized trial. *J Cardiothorac Vasc Anesth* 2010;24:790-796.
91. Schultz JE, Hsu AK, Nagase H, Gross GJ. TAN-67, a  $\delta_1$ -opioid receptor agonist, reduces infarct size via activation of  $G_{i/o}$  proteins and  $K_{ATP}$  channels. *Am J Physiol* 1998;274:H909-H914.
92. Kevelaitis E, Peynet J, Mouas C, Launay JM, Menasche P. Opening of potassium channels: the common cardioprotective link between preconditioning and natural hibernation? *Circulation* 1999;99:3079-3085.



93. Valtchanova-Matchouganska A, Ojewole JA. Involvement of opioid delta ( $\delta$ )- and kappa ( $\kappa$ )-receptors in ischemic preconditioning in a rat model of myocardial infarction. *Methods Find Exp Clin Pharmacol* 2002;24:139-144.
94. Ikeda Y, Miura T, Sakamoto J, Miki T, Tanno M, Kobayashi H, Ohori K, Takahashi A, Shimamoto K. Activation of ERK and suppression of calcineurin are interacting mechanisms of cardioprotection afforded by  $\delta$ -opioid receptor activation. *Basic Res Cardiol* 2006;101:418-426.
95. Seymour EM, Wu SY, Kovach MA, Romano MA, Traynor JR, Claycomb WC, Bolling SF. HL-1 myocytes exhibit PKC and  $K_{ATP}$  channel-dependent delta opioid preconditioning. *J Surg Res* 2003;114:187-194.
96. Gross ER, Hsu AK, Gross GJ. Acute methadone treatment reduces myocardial infarct size via the  $\delta$ -opioid receptor in rats during reperfusion. *Anesth Analg* 2009;109:1395-1402.
97. Takasaki Y, Wolff RA, Chien GL, van Winkle DM. Met<sup>5</sup>-enkephalin protects isolated adult rabbit cardiomyocytes via  $\delta$ -opioid receptors. *Am J Physiol* 1999;277:H2442-H2450.
98. Cao Z, Liu L, Van Winkle DM. Activation of  $\delta$ - and  $\kappa$ -opioid receptors by opioid peptides protects cardiomyocytes via  $K_{ATP}$  channels. *Am J Physiol Heart Circ Physiol* 2003;285:H1032-H1039.
99. Kuzume K, Wolff RA, Amakawa K, Kuzume K, Van Winkle DM. Sustained exogenous administration of Met<sup>5</sup>-enkephalin protects against infarction in vivo. *Am J Physiol Heart Circ Physiol*. 2003;285:H2463-H2470.
100. Kuzume K, Kuzume K, Cao Z, Liu L, Van Winkle DM. Long-term infusion of Met<sup>5</sup>-enkephalin fails to protect murine hearts against ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2005;288:H1717-H1723.
101. Sigg DC, Coles JA, Oeltgen PR, Iaizzo PA. Role of  $\delta$ -opioid receptor agonists on infarct size reduction in swine. *Am J Physiol Heart Circ Physiol* 2002;282:H1953-H1960.
102. Fryer RM, Hsu AK, Nagase H, Gross GJ. Opioid-induced cardioprotection against myocardial infarction and arrhythmias: mitochondrial versus sarcolemmal ATP-sensitive potassium channels. *J Pharmacol Exp Ther* 2000;294:451-457.
103. Maslov LN, Platonov AA, Lasukova TV, Lishmanov IuB, Oeltgen P, Nagase H, Podoksenov IuK, Podoksenov AIu. Delta-opioid receptor activation prevents appearance of irreversible damages of cardiomyocytes and exacerbates myocardial contractility dysfunction during ischemia and reperfusion. *Patol Fiziol Eksp Ter* 2006;(4):13-17.
104. Platonov AA, Lasukova TV, Maslov LN, Downey JM, Nagaze H, Ovchinnikov MV. Delta-opioid receptor agonists prevent the irreversible damage of cardiomyocytes in ischemised-reperfused isolated rat heart. *Eksp Klin Farmakol* 2004;67:26-29.

105. Huh J, Gross GJ, Nagase H, Liang BT. Protection of cardiac myocytes via  $\delta_1$ -opioid receptors, protein kinase C, and mitochondrial  $K_{ATP}$  channels. *Am J Physiol Heart Circ Physiol* 2001;280:H377-H383.
106. Lasukova TV, Maslov LN, Platonov AA, Lishmanov IuB, Oeltgen P. The effect of stimulation of cardiac  $\delta_1$ -opioid receptors on the resistance of isolated heart to the action of ischemia and reperfusion. *Eksp Klin Farmakol* 2005;68: 9-24.
107. McPherson BC, Yao Z. Morphine mimics preconditioning via free radical signals and mitochondrial  $K_{ATP}$  channels in myocytes. *Circulation* 2001;103:290-295.
108. Gross ER, Hsu AK, Gross GJ. GSK3 $\beta$  inhibition and  $K_{ATP}$  channel opening mediate acute opioid-induced cardioprotection at reperfusion. *Basic Res Cardiol* 2007;102:341-349.
109. Peart JN, Patel HH, Gross GJ. Delta-opioid receptor activation mimics ischemic preconditioning in the canine heart. *J Cardiovasc Pharmacol* 2003;42:78-81.
110. Maslov LN, Barzakh EI, Krylatov AV, Brown SA, Oeltgen PR, Govindaswami M, Chernysheva GA, Solenkova NV, Lishmanov AIu, Tsibul'nikov SIu, Krieg T, Zhang E. Significance of opioid receptors in regulation of cardiac tolerance in pathogenic impact of long-term ischemia-reperfusion in vivo. *Russ Fiziol Zh Im I M Sechenova*. 2009;95:563-572.
111. Maslov LN, Lishmanov YB, Oeltgen PR, Barzakh EI, Krylatov AV, Naryzhnaya NV, Pei JM, Brown SA. Comparative analysis of the cardioprotective properties of opioid receptor agonists in a rat model of myocardial infarction. *Acad Emerg Med* 2010;17:1239-1246.
112. Lasukova TV, Maslov LN, Lishmanov YuB, Gross GJ. Role of  $\delta_1$ -opiate receptors in regulation of contractility in isolated rat heart during normal oxygenation and ischemia/reperfusion. *Bull Exp Biol Med* 2004; 31: 80-86.
113. Lasukova TV, Maslov LN, Gorbunov AC, Tsibul'nikov SIu. Intracellular mechanisms of opioidergic regulation of the myocardial function during normoxia and postischemic reperfusion. *Russ Fiziol Zh Im I M Sechenova* 2009;95:376-386.
114. Lishmanov YuB, Maslov LN, Lasukova TV, Platonov AA, Oeltgen P. Activation of  $\delta$ -opioid receptors increases resistance of isolated heart to ischemia/reperfusion: The role of cAMP and intracellular calcium. *Bull Exp Biol Med* 2005;32:45-51.
115. Watson MJ, Holt JD, O'Neill SJ, Wei K, Pendergast W, Gross GJ, Gengo PJ, Chang KJ. ARD-353 [4-((2R,5S)-4-(R)-(4-diethylcarbamoylphenyl)(3-hydroxyphenyl)methyl)-2,5-dimethylpiperazin-1-ylmethyl)benzoic acid], a novel nonpeptide delta receptor agonist, reduces myocardial infarct size without central effects. *J Pharmacol Exp Ther* 2006;316:423-430.
116. Gross ER, Hsu AK, Gross GJ. The JAK/STAT pathway is essential for opioid-induced cardioprotection: JAK2 as a mediator of STAT3, Akt, and GSK-3 $\beta$ . *Am J Physiol Heart Circ Physiol* 2006;291:H827-H834.

117. Maslov LN, Lishmanov YB, Oeltgen PR, Barzakh EI, Krylatov AV, Govindaswami M, Brown SA. Activation of peripheral  $\delta_2$  opioid receptors increases cardiac tolerance to ischemia/reperfusion injury. Involvement of protein kinase C, NO-synthase,  $K_{ATP}$  channels and the autonomic nervous system. *Life Sci* 2009;84:657-663.
118. Wu S, Li H.Y, Wong TM. Cardioprotection of preconditioning by metabolic inhibition in the rat ventricular myocyte. Involvement of  $\kappa$ -opioid receptor. *Circ Res* 1999;84:1388-1395.
119. Lasukova TV, Maslov LN, Platonov AA, Guzarova NV, Lishmanov YuB. Cardioprotective effect of  $\kappa_1$ -opioid receptor activation and role of cAMP in its realization. *Bull Exp Biol Med* 2007;143:22-25.
120. Lasukova TV, Maslov LN, Platonov AA, Guzarova NV, Lishmanov IuB. Role of  $\kappa_1$  opioid receptors and cAMP in regulation of cardiac tolerance to ischemia and reperfusion. *Bull Exp Biol Med*. 2008;35:499-506.
121. Peart JN, Gross ER, Gross GJ. Effect of exogenous kappa-opioid receptor activation in rat model of myocardial infarction. *J Cardiovasc Pharmacol* 2004;43:410-415.
122. Schindler CW, Graczyk Z, Gilman JP, Negus SS, Bergman J, Mello NK, Goldberg SR. Effects of kappa opioid agonists alone and in combination with cocaine on heart rate and blood pressure in conscious squirrel monkeys. *Eur J Pharmacol* 2007;576:107–113.
123. Wang Q, Sun Y, Li J, Xing W, Zhang S, Gu X, Feng N, Zhao L, Fan R, Wang Y, Yin W, Pei J. Quaternary ammonium salt of U50488H, a new  $\kappa$ -opioid receptor agonist, protects rat heart against ischemia/reperfusion injury. *Eur J Pharmacol* 2014;737:177-184.
124. Lasukova TV, Rebrova TY, Tam SV. Activation of mu-opiate receptors as a factor of regulation of heart resistance to ischemia-reperfusion and oxidative stress. *Bull Exp Biol Med* 2000;130:752-755.
125. Lasukova TV, Maslov LN, Lishmanov IuB, Tam SV, Gross GJ. Effect of stimulation of  $\mu$ -opiate receptors on contractility of the isolated heart in normal oxygenation and during ischemia-reperfusion. *Russ Fiziol Zh Im I M Sechenova* 2001;87:649-658.
126. Maslov LN, Lasukova TV, Solenkova NV, Lishmanov AIu, Bogomaz SA, Tam SV, Gross GJ. Participation of  $K_{ATP}$ -channels in cardioprotective effect of mu-opioid receptor agonists in acute ischemia and reperfusion of the isolated heart. *Eksp Klin Farmakol* 2001;64:23-27.
127. Lishmanov IuB, Maslov LN, Naumova AV, Bogomaz SA.  $\mu$ -Opiate receptor activation and increase in heart resistance to ischemic and reperfusion injury. *Russ Fiziol Zh Im I M Sechenova* 1998;84:1223-1230.
128. Schultz JE, Hsu AK, Gross GJ. Ischemic preconditioning in the intact rat heart is mediated by  $\delta_1$ - but not  $\mu$ - or  $\kappa$ -opioid receptors. *Circulation* 1998;97:1282-1289.

129. Gross GJ, Hsu A, Nithipatikom K, Bobrova I, Bissessar E. Eribis peptide 94 reduces infarct size in rat hearts via activation of centrally located  $\mu$  opioid receptors. *J Cardiovasc Pharmacol* 2012;59:194-197.
130. Kistner JR, Miller ED, Lake CL, Ross WT. Indices of myocardial oxygenation during coronary-artery revascularization in man with morphine versus halothane anesthesia. *Anesthesiology* 1979;50:324-330.
131. Kisin I, Markiewicz W, Birkhahn J. Effect of large doses of morphine on experimental myocardial ischemia in cats. *Isr J Med Sci* 1979;15:588-591.
132. Markiewicz W, Finberg JPM, Lichtig C. Morphine increases myocardial infarct size in rats. *Anesth Analg* 1982;61:843-846.
133. Lee AY, Wong TM. Naloxone reduces release of creatine kinase in the isolated ischemic rat heart. *Proc Soc Exp Biol Med* 1985;179:219-221.
134. Lahti RA, Mickelson MM, McCall JM, Von Voigtlander PF [ $^3$ H]U-69593 a highly selective ligand for the opioid  $\kappa$  receptor. *Eur J Pharmacol* 1985;109:281-284.
135. Roques BP, Gacel G, Dauge V, Baamonde A, Calenco G, Turcaud S, Coric P, Fournie-Zaluski MC. Novel approaches in the development of new analgesics. *Neurophysiol Clin* 1990;20:369-387.
136. Chen Z, Li T, Zhang B. Morphine postconditioning protects against reperfusion injury in the isolated rat hearts. *J Surg Res* 2008;145: 287-294.
137. Heijna MH, Padt M, Hogenboom F, Portoghese PS, Mulder AH, Schoffelmeer AN. Opioid receptor-mediated inhibition of dopamine and acetylcholine release from slices of rat nucleus accumbens, olfactory tubercle and frontal cortex. *Eur J Pharmacol* 1990;181:267-278.
138. Maslov LN, Lishmanov IuB, Headrick JP, Pei J-M, Hanus L, Krylatov AV, Naryzhnaia NV. Perspective of creation of drugs on basis of opioids increasing cardiac tolerance to pathogenic impact of ischemia reperfusion. *Eksp Klin Farmakol* 2012;75:22-28.
139. Kazmierski W, Wire WS, Lui GK, Knapp RJ, Shook JE, Burks TF, Yamamura HI, Hruby VJ. Design and synthesis of somatostatin analogues with topographical properties that lead to highly potent and specific  $\mu$  opioid receptor antagonists with greatly reduced binding at somatostatin receptors. *J Med Chem* 1988;31:2170-2177.
140. Wang TL, Huang YH, Chang H. Somatostatin analogue mimics acute ischemic preconditioning in a rat model of myocardial infarction. *J Cardiovasc Pharmacol* 2005;5:327-332.
141. Peart JN, Gross GJ. Exogenous activation of  $\delta$ - and  $\kappa$ -opioid receptors affords cardioprotection in isolated murine heart. *Basic Res Cardiol* 2004;99:29-37.

142. Aitchison KA, Baxter GF, Awan MM, Smith RM, Yellon DM, Opie LH. Opposing effects on infarction of delta and kappa opioid receptor activation in the isolated rat heart: implications for ischemic preconditioning. *Basic Res Cardiol*. 2000;95:1-10.
143. Rothman RB, Bykov V, Xue BG, Xu H, De Costa BR, Jacobson AE, Rice KC, Kleiman JE, Brady LS. Interaction of opioid peptides and other drugs with multiple kappa receptors in rat and human brain. Evidence for species differences. *Peptides* 1992;13:977-987.
144. Meine TJ, Roe MT, Chen AY, Patel MR, Washam JB, Ohman EM, Peacock WF, Pollack CV, Gibler WB, Peterson ED; CRUSADE Investigators. Association of intravenous morphine use and outcomes in acute coronary syndromes: results from the CRUSADE Quality Improvement Initiative. *Am Heart J* 2005;149:1043-1049.
145. Conahan TJ, Ominsky AJ, Wollman H, Stroth RA. A prospective random comparison of halothane and morphine for open-heart anesthesia: one year's experience. *Anesthesiology* 1973;38:528-535.
146. Lappas DG, Buckley MJ, Laver MB, Daggett WM, Lowenstein E. Left ventricular performance and pulmonary circulation following addition of nitrous oxide to morphine during coronary-artery surgery. *Anesthesiology* 1975;43:61-69.
147. Van de Werf F, Ardissino D, Betriu A, Cokkinos DV, Falk E, Fox KA, Julian D, Lengyel M, Neumann FJ, Ruzyllo W, Thygesen C, Underwood SR, Vahanian A, Verheugt FW, Wijns W. Task Force on the Management of Acute Myocardial Infarction of the European Society of Cardiology. Management of acute myocardial infarction in patients presenting with ST-segment elevation. The Task Force on the Management of Acute Myocardial Infarction of the European Society of Cardiology. *Eur Heart J* 2003;24:28-66.
148. Metelitsa V.I. Handbook on Clinical Pharmacology of Cardiovascular Drugs. Moscow, Medpraktika. 1996; pp.784.
149. Stanley TH, Gray NH, Stanford W, Armstrong R. The effects of high-dose morphine on fluid and blood requirements in open-heart operations. *Anesthesiology* 1973;38:536-541.
150. Kulbertus H, Pierard L. Myocardial Infarction. In: Jeffers JD, Fulvio B, editors. *Cardiology*. McGraw-Hill International; 1999. p 386.
151. Kettler D, Hilfiker O, Sonntag H. Narcotics and the coronary circulation. In: Estafanous FG, editor. *Opioids in Anesthesia*. Boston, London: Butterworth Publishers; 1991. p 194-201.
152. Khan MG. *Cardiac Drug Therapy*. Seventh edition. Totowa, New Jersey: Humana Press; 2007. p 186,187,236.
153. Zweier JL, Talukder MA. The role of oxidants and free radicals in reperfusion injury. *Cardiovasc Res* 2006;70:181-190.
154. Webster KA. Programmed death as a therapeutic target to reduce myocardial infarction. *Trends Pharmacol Sci* 2007;28:492-499.

155. Halestrap AP, Clarke SJ, Khaliulin I. The role of mitochondria in protection of the heart by preconditioning. *Biochim Biophys Acta* 2007;1767:1007-1031.
156. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 2007;87:99-163.
157. Hausenloy DJ, Ong SB, Yellon DM. The mitochondrial permeability transition pore as a target for preconditioning and postconditioning. *Basic Res Cardiol* 2009;104:189-202.
158. Liu H, Zhang HY, McPherson BC, Baman T, Roth S, Shao Z, Zhu X, Yao Z. Role of opioid  $\delta_1$  receptors, mitochondrial  $K_{ATP}$  channels, and protein kinase C during cardiocyte apoptosis. *J Mol Cell Cardiol* 2001;33:2007-2014.
159. Okubo S, Tanabe Y, Takeda K, Kitayama M, Kanemitsu S, Kukreja RC, Takekoshi N. Ischemic preconditioning and morphine attenuate myocardial apoptosis and infarction after ischemia-reperfusion in rabbits: role of  $\delta$ -opioid receptor. *Am J Physiol Heart Circ Physiol* 2004;287:H1786-H1791.
160. Barrere-Lemaire S, Combes N, Sportouch-Dukhan C, Richard S, Nargeot J, Piot C. Morphine mimics the antiapoptotic effect of preconditioning via an  $Ins(1,4,5)P_3$  signaling pathway in rat ventricular myocytes. *Am J Physiol Heart Circ Physiol* 2005;288:H83-H88.
161. Li L, Zhang H, Li T, Zhang B. Involvement of adenosine monophosphate-activated protein kinase in morphine-induced cardioprotection. *J Surg Res* 2011;169:179-187.
162. Kim HS, Kim SY, Kwak YL, Hwang KC, Shim YH. Hyperglycemia attenuates myocardial preconditioning of remifentanyl. *J Surg Res* 2012;174:231-237.
163. Rong F, Peng Z, Ye MX, Zhang QY, Zhao Y, Zhang SM, Guo HT, Hui B, Wang YM, Liang C, Gu CH, Tao C, Cui Q, Yu SQ, Yi DH, Pei JM. Myocardial apoptosis and infarction after ischemia/reperfusion are attenuated by  $\kappa$ -opioid receptor agonist. *Arch Med Res* 2009;40:227-234.
164. Fryer RM, Hsu AK, Eells JT, Nagase H, Gross GJ. Opioid-induced second window of cardioprotection: potential role of mitochondrial  $K_{ATP}$  channels. *Circ Res* 1999;84:846-851.
165. Fryer RM, Hsu AK, Gross GJ. ERK and p38 MAP kinase activation are components of opioid-induced delayed cardioprotection. *Basic Res Cardiol* 2001;96:136-142.
166. Guo Y, Stein AB, Wu WJ, Zhu X, Tan W, Li Q, Bolli R. Late preconditioning induced by NO donors, adenosine  $A_1$  receptor agonists, and  $\delta_1$ -opioid receptor agonists is mediated by iNOS. *Am J Physiol Heart Circ Physiol* 2005;289:H2251-H2257.
167. Patel HH, Hsu A, Gross GJ. Delayed cardioprotection is mediated via a non-peptide  $\delta$  opioid agonist, SNC-121, independent of opioid receptor stimulation. *Basic Res Cardiol* 2004;99:38-45.
168. Shinmura K, Nagai M, Tamaki K, Bolli R. Gender and aging do not impair opioid-induced late preconditioning in rats. *Basic Res Cardiol* 2004;99:46-55.

169. Frassdorf J, Weber NC, Obal D, Toma O, Mullenheim J, Kojda G, Preckel B, Schlack W. Morphine induces late cardioprotection in rat hearts *in vivo*: the involvement of opioid receptors and nuclear transcription factor  $\kappa$ B. *Anesth Analg* 2005;101:934-941.
170. Jiang X, Shi E, Nakajima Y, Sato S. COX-2 mediates morphine-induced delayed cardioprotection via an iNOS-dependent mechanism. *Life Sci* 2006;78:2543-2549.
171. Zhou JJ, Pei JM, Wang GY, Wu S, Wang WP, Cho CH, Wong T.M. Inducible HSP70 mediates delayed cardioprotection via U-50488H pretreatment in rat ventricular myocytes. *Am J Physiol Heart Circ Physiol* 2001;281:H40-H47.
172. Chen M, Zhou JJ, Kam KW, Qi JS, Yan WY, Wu S, Wong TM. Roles of  $K_{ATP}$  channels in delayed cardioprotection and intracellular  $Ca^{2+}$  in the rat heart as revealed by  $\kappa$ -opioid receptor stimulation with U50488H. *Br J Pharmacol* 2003;140:750-758.
173. Wang GY, Zhou JJ, Shan J, Wong TM. Protein kinase C-epsilon is a trigger of delayed cardioprotection against myocardial ischemia of kappa-opioid receptor stimulation in rat ventricular myocytes. *J Pharmacol Exp Ther* 2001;299(2):603-610.
174. Yu CK, Li YH, Wong GT, Wong TM, Irwin MG. Remifentanil preconditioning confers delayed cardioprotection in the rat. *Br J Anaesth* 2007;99:632-638.
175. Sun HT, Xue FS, Liu KP, Sun L, Xu YC, Liao X, Yang QY, Zhang YM. Effect of remifentanil preconditioning on myocardial ischemia-reperfusion injury. *Acta Acad Med Sci* 2009;31:612-615.
176. Dickson EW, Blehar DJ, Carraway RE, Heard SO, Steinberg G, Przyklenk K. Naloxone blocks transferred preconditioning in isolated rabbit hearts. *J Mol Cell Cardiol* 2001; 33: 1751-1756.
177. Dickson EW, Tubbs RJ, Porcaro WA, Lee WJ, Blehar DJ, Carraway RE, Darling CE, Przyklenk K. Myocardial preconditioning factors evoke mesenteric ischemic tolerance via opioid receptors and  $K_{ATP}$  channels. *Am. J. Physiol. Heart Circ. Physiol.* 2002; 283(1): H22-H28.
178. Dickson EW, Ludwig PS, Ackermann LW, Buresh CT, Denning GM. Met<sup>5</sup>-enkephalin-Arg<sup>6</sup>-Phe<sup>7</sup> (MEAP): a cardioprotective hormonal opioid. *Acad. Emerg. Med.* 2006;13:813-819.
179. Patel HH, Moore J, Hsu AK, Gross GJ. Cardioprotection at a distance: mesenteric artery occlusion protects the myocardium via an opioid sensitive mechanism. *J Mol Cell Cardiol* 2002;34:1317-1323.
180. Weinbrenner C, Schulze F, Sarvary L, Strasser RH. Remote preconditioning by infrarenal aortic occlusion is operative via  $\delta$ 1-opioid receptors and free radicals *in vivo* in the rat heart. *Cardiovasc Res* 2004;61:591-599.
181. Zhang SZ, Wang NF, Xu J, Gao Q, Lin GH, Bruce IC, Xia Q. Kappa-opioid receptors mediate cardioprotection by remote preconditioning. *Anesthesiology* 2006;105:550-556.

182. Rehni AK, Singh N, Jaggi AS. Possible involvement of insulin, endogenous opioids and calcitonin gene-related peptide in remote ischaemic preconditioning of the brain. *Yakugaku Zasshi*. 2007;127:1013-1020.
183. Rentoukas I, Giannopoulos G, Kaoukis A, Kossyvakis C, Raisakis K, Driva M, Panagopoulou V, Tsarouchas K, Vavetsi S, Pyrgakis V, Deftereos S. Cardioprotective role of remote ischemic preconditioning in primary percutaneous coronary intervention: enhancement by opioid action. *JACC Cardiovasc Interv* 2010;3:49-55.
184. Hiranuma T, Kitamura K, Taniguchi T, Kanai M, Arai Y, Iwao K, Oka T. Protection against dynorphin-(1-8) hydrolysis in membrane preparations by the combination of amastatin, captopril and phosphoramidon. *J Pharmacol Exp Ther* 1998;286:863-869.
185. Hiranuma T, Kitamura K, Taniguchi T, Kobayashi T, Tamaki R, Kanai M, Akahori K, Iwao K, Oka T. Effects of three peptidase inhibitors, amastatin, captopril and phosphoramidon, on the hydrolysis of [Met<sup>5</sup>]-enkephalin-Arg<sup>6</sup>-Phe<sup>7</sup> and other opioid peptides. *Naunyn-Schmiedeberg's Arch Pharmacol* 1998;357:276-282.
186. Chang WL, Lee SS, Su MJ. Attenuation of post-ischemia reperfusion injury by thaliporphine and morphine in rat hearts. *J Biomed Sci* 2005;12:611-619.
187. Tsutsumi YM, Yokoyama T, Horikawa Y, Roth DM, Patel HH. Reactive oxygen species trigger ischemic and pharmacological preconditioning: *in vivo* and *in vitro* characterization. *Life Sci* 2007;81:1223-1227.
188. Jang Y, Xi J, Wang H, Mueller RA, Norfleet EA, Xu Z. Postconditioning prevents reperfusion injury by activating  $\delta$ -opioid receptors. *Anesthesiology* 2008;108:243-250.
189. Mourouzis I, Saranteas T, Perimenis P, Tesseromatis C, Kostopanagiotou G, Pantos C, Cokkinos DV. Morphine administration at reperfusion fails to improve postischemic cardiac function but limits myocardial injury probably via heat-shock protein 27 phosphorylation. *Eur J Anaesthesiol* 2009;26:572-581.
190. Huhn R, Heinen A, Weber NC, Schlack W, Preckel B, Hollmann MW. Ischaemic and morphine-induced post-conditioning: impact of mK<sub>Ca</sub> channels. *Br J Anaesth* 2010;105:589-595.
191. Kim JH, Chun KJ, Park YH, Kim J, Kim JS, Jang YH, Lee MY, Park JH. Morphine-induced preconditioning modulates mitochondrial permeability transition pore opening via delta-1 opioid receptors activation in isolated rat hearts. *Korean J Anesthesiol* 2011;61:69-74.
192. Peart JN, Gross ER, Reichelt ME, Hsu A, Headrick JP, Gross GJ. Activation of kappa-opioid receptors at reperfusion affords cardioprotection in both rat and mouse hearts. *Basic Res Cardiol* 2008;103:454-463.
193. Wong GT, Li R, Jiang LL, Irwin MG. Remifentanyl post-conditioning attenuates cardiac ischemia-reperfusion injury via  $\kappa$  or  $\delta$  opioid receptor activation. *Acta Anaesthesiol Scand* 2010;54(4):510-518.



194. Chun KJ, Park YH, Kim JS, Jang Y, Kim JH, Kim J, Lee MY. Comparison of 5 different remifentanyl strategies against myocardial ischemia-reperfusion injury. *J Cardiothorac Vasc Anesth* 2011;25:926-930.
195. Karlsson LO, Grip L, Bissessar E, Bobrova I, Gustafsson T, Kavianipour M, Odenstedt J, Wikstrom G, Gonon AT. Opioid receptor agonist Eribis peptide 94 reduces infarct size in different porcine models for myocardial ischaemia and reperfusion. *Eur J Pharmacol* 2011; 651:146-151.
196. Karlsson LO, Bergh N, Li L, Bissessar E, Bobrova I, Gross GJ, Akyurek LM, Grip L. Dose-dependent cardioprotection of enkephalin analogue Eribis peptide 94 and cardiac expression of opioid receptors in a porcine model of ischaemia and reperfusion. *Eur J Pharmacol* 2012;674:378-383.
197. Wu Y, Gu EW, Zhu Y, Zhang L, Liu XQ, Fang WP. Sufentanil limits the myocardial infarct size by preservation of the phosphorylated connexin 43. *Int Immunopharmacol* 2012;13:341-346.
198. Emmerson PJ, Liu M-R, Woods JH, Medzihradsky F. Binding affinity and selectivity of opioids at mu, delta and kappa receptors in monkey brain membranes. *J. Pharmacol. Exp. Ther.* 1994; 271: 1630-1637.
199. Chen QL, Gu EW, Zhang L, Cao YY, Zhu Y, Fang WP. Diabetes mellitus abrogates the cardioprotection of sufentanil against ischaemia/reperfusion injury by altering glycogen synthase kinase-3 $\beta$ . *Acta Anaesthesiol Scand* 2013;57:236-242.
200. Ha JY, Lee YC, Park SJ, Jang YH, Kim JH. Remifentanyl postconditioning has cross talk with adenosine receptors in the ischemic-reperfused rat heart. *J Surg Res* 2015;195:37-43.
201. Knapp RJ, Landsman R, Waite S, Malatynska E, Varga E, Haq W, Hruby V, Roeske WR, Nagase H, Yamamura HJ. Properties of TAN-67, a nonpeptidic  $\delta$ -opioid receptor agonist, at cloned human  $\delta$ - and  $\mu$ -opioid receptors. *Eur J Pharmacol* 1995;291:129-134.
202. Groban L, Vernon JC, Butterworth J. Intrathecal morphine reduces infarct size in a rat model of ischemia-reperfusion injury. *Anesth Analg* 2004;98:903-909.
203. Li R, Wong GT, Wong TM, Zhang Y, Xia Z, Irwin MG. Intrathecal morphine preconditioning induces cardioprotection via activation of delta, kappa, and mu opioid receptors in rats. *Anesth Analg* 2009;108:23-29.
204. Wong GT, Ling Ling J, Irwin MG. Activation of central opioid receptors induces cardioprotection against ischemia-reperfusion injury. *Anesth Analg* 2010;111:24-28.
205. Ling Ling J, Wong GT, Yao L, Xia Z, Irwin MG. Remote pharmacological post-conditioning by intrathecal morphine: cardiac protection from spinal opioid receptor activation. *Acta Anaesthesiol Scand* 2010;54:1097-1104.

206. Lu Y, Dong C, Yu J, Li L. Erratum to: Role of central and peripheral opioid receptors in the cardioprotection of intravenous morphine preconditioning. *Ir J Med Sci.* 2015 Dec 18. Accept in press.
207. Wong GT, Yao L, Xia Z, Irwin MG. Intrathecal morphine remotely preconditions the heart via a neural pathway. *J Cardiovasc Pharmacol* 2012;60:172-178.
208. Lu Y, Hu J, Zhang Y, Dong C. Spinal neuronal NOS activation mediates intrathecal fentanyl preconditioning induced remote cardioprotection in rats. *Int Immunopharmacol* 2014;19:127-131.
209. Polonskii VM, Yarygin KN, Krovosheev OG, Moskovkin GN, Vinogradov VA. Effect of the antiulcerative action (central or peripheral) of the synthetic enkephalin analog dalargin in experimental cysteamine-induced duodenal ulcer in rats. *Bull Exp Biol Med* 1987;103(4):488-490.
210. Varga EV, Li X, Stropova D, Zalewska T, Landsman RS, Knapp RJ, Malatynska E, Kawai K, Mizusura A, Nagase H, Calderon SN, Rice K, Hruby VJ, Roeske WR, Yamamura HI. The third extracellular loop of the human  $\delta$ -opioid receptor determines the selectivity of  $\delta$ -opioid agonists. *Molecular Pharmacology* 1996;50:1619-1624.
211. Szabo B, Hedler L, Ensinger H, Starke K. Opioid peptides decrease noradrenaline release and blood pressure in the rabbit at peripheral receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 1986;332:50-56.
212. Benedict PE, Benedict MB, Su TP, Bolling SF. Opiate drugs and  $\delta$ -receptor-mediated myocardial protection. *Circulation* 1999;100:II357-II360.
213. Shi E, Jiang X, Bai H, Gu T, Chang Y, Wang J. Cardioprotective effects of morphine on rat heart suffering from ischemia and reperfusion. *Chin Med J (Engl)* 2003;116:1059-1062.
214. Kato R, Foex P. Fentanyl protects the heart against ischaemic injury via opioid receptors, adenosine  $A_1$  receptors and  $K_{ATP}$  channel linked mechanisms in rats. *Br J Anaesth* 2000;84:204-214.
215. Kato R, Foex P. Fentanyl reduces infarction but not stunning via  $\delta$ -opioid receptors and protein kinase C in rats. *Br J Anaesth.* 2000;84:608-614.
216. Pyle WG, Smith TD, Hofmann PA. Cardioprotection with  $\kappa$ -opioid receptor stimulation is associated with a slowing of cross-bridge cycling. *Am J Physiol Heart Circ Physiol* 2000;297:H1941-H1948.
217. De Costa BR, Bowen WD, Hellewell SB, George C, Rothman RB, Reid AA, Walker JM, Jacobson AE, Rice KC. Alterations in the stereochemistry of the  $\kappa$ -selective opioid agonist U50,488 results in high-affinity  $\sigma$  ligands. *J Med Chem* 1989;32:1996-2002.
218. Townsend D, Brown DR. Characterization of specific  $\delta$ -opioid binding sites in the distal small intestine of swine. *Eur J Pharmacol* 2003;482:111-116.

219. Poonyachoti S, Portoghese PS, Brown DR. Characterization of opioid receptors modulating neurogenic contractions of circular muscle from porcine ileum and evidence that delta- and kappa-opioid receptors are coexpressed in myenteric neurons. *J Pharmacol Exp Ther* 2001;297:69-77.
220. Genade S, Moolman JA, Lochner A. Opioid receptor stimulation acts as mediator of protection in ischaemic preconditioning. *Cardiovasc J S Afr* 2001;12:8-16.
221. Gacel G, Fournie-Zaluski M-C, Fellion E, Roques BP. Evidence of the preferential involvement of  $\mu$  receptors in analgesia using enkephalins highly selective for peripheral  $\mu$  or  $\delta$  receptors. *J Med Chem* 1981;24:1119-1124.
222. Bolling SF, Badhwar V, Schwartz CF, Oeltgen PR, Kilgore K, Su TP. Opioids confer myocardial tolerance to ischemia: interaction of delta opioid agonists and antagonists. *J Thorac Cardiovasc Surg* 2001;122:476-481.
223. Rothman RB, Xu H, Char GU, Kim A, De Costa BR, Rice KC, Zimmerman DM. Phenylpiperidine opioid antagonists that promote weight loss in rats have high affinity for the  $\kappa_{2B}$  (enkephalin-sensitive) binding site. *Peptides* 1993;14:17-20.
224. Sigg DC, Coles JA, Gallagher WJ, Oeltgen PR, Iaizzo PA. Opioid preconditioning: myocardial function and energy metabolism. *Ann Thorac Surg* 2001;72:1576-1582.
225. Shinmura K, Nagai M, Tamaki K, Tani M, Bolli R. COX-2-derived prostacyclin mediates opioid-induced late phase of preconditioning in isolated rat hearts. *Am J Physiol Heart Circ Physiol* 2002;283:H2534-H2543.
226. Wu D, Soong Y, Zhao GM, Szeto HH. A highly potent peptide analgesic that protects against ischemia-reperfusion-induced myocardial stunning. *Am J Physiol Heart Circ Physiol* 2002;283:H783-H791.
227. Schiller PW, Nguyen TM, Berezowska I, Dupuis S, Weltrowska G, Chung NN, Lemieux C. Synthesis and in vitro opioid activity profiles of DALDA analogues. *Eur J Med Chem* 2000;35:895-901.
228. Peart JN, Gross ER, Headrick JP, Gross GJ. Impaired p38 MAPK/HSP27 signaling underlies aging-related failure in opioid-mediated cardioprotection. *J Mol Cell Cardiol* 2007;42:972-980.
229. Romano MA, McNish R, Seymour EM, Traynor JR, Bolling SF. Differential effects of opioid peptides on myocardial ischemic tolerance. *J Surg Res* 2004;119:46-50.
230. Chen BP, Mao HJ, Fan FY, Bruce IC, Xia Q. Delayed uncoupling is related to cardioprotection induced by  $\kappa$ -agonist U-50,488H in rat heart. *Scand Cardiovasc J* 2005;39:375-382.
231. Shan Y, Sun S, Yang X, Weil MH, Tang W. Opioid receptor agonist reduces myocardial ischemic injury when administered during early phase of myocardial ischemia. *Resuscitation* 2010;81:761-765.

232. Cheng L, Ma S, Wei LX, Guo HT, Huang LY, Bi H, Fan R, Li J, Liu YL, Wang YM, Sun X, Zhang QY, Yu SQ, Yi DH, Ma XL, Pei JM. Cardioprotective and antiarrhythmic effect of U50,488H in ischemia/reperfusion rat heart. *Heart Vessels* 2007;22:335-344.
233. Grosse Hartlage MA, Theisen MM, Monteiro de Oliveira NP, Van Aken H, Fobker M, Weber TP.  $\kappa$ -Opioid receptor antagonism improves recovery from myocardial stunning in chronically instrumented dogs. *Anesth Analg* 2006;103:822-832.
234. Panova EI. Short-term and long-term prognosis in patients with myocardial infarction. *Klin Med* 2008;86:19-23.
235. Fagbemi O, Kane KA, Lepran J, Parratt JR, Szekeres L. Anti-arrhythmic actions of meptazinol, a partial agonist at opiate receptors, in acute myocardial ischemia. *Br J Pharmacol* 1983;78:455-460.
236. Saini V, Carr DB, Verrier RL. Comparative effects of the opioids fentanyl and buprenorphine on ventricular vulnerability during acute coronary artery occlusion. *Cardiovasc Res* 1989;23:1001-1006.
237. Hess L, Vrana M, Vranova Z, Fejfar Z. The antifibrillatory effect of fentanyl, sufentanil and carfentanil in acute phase of local myocardial ischemia in the dog. *Acta Cardiol* 1989;44:303-311.
238. Hansen DD, Hickey PR. Anesthesia for hypoplastic left heart syndrome: use of high dose fentanyl in 30 neonatans. *Anesth Analg* 1986;65:127-132.
239. Maslov LN, Lishmanov YuB. The anti-arrhythmic effect of D-Ala<sup>2</sup>,Leu<sup>5</sup>,Arg<sup>6</sup>-enkephalin and its possible mechanism. *Int J Cardiol* 1993;40:89-94.
240. Mikhailova SD, Semushkina TM, Bebiakova NA. Effect of dalargin on the course of myocardial ischemia. *Kardiologiya* 1991;31:13-15.
241. Grekova TI, Reznikov KM, Vinokurova OV, Kireeva AA, Taratinova TI, Nikolaevskii VA, Shchetinkina NA. The effect of dalargin on the course of experimental cardiac arrhythmias. *Eksp Klin Farmakol* 1994;57:24-26.
242. Lishmanov YuB, Maslov LN, Naryzhnaya NV, Tam SW. Ligands for opioid and  $\sigma$ -receptors improve cardiac electrical stability in rat models of post-infarction cardiosclerosis and stress. *Life Sci* 1999;65:PL13-PL17.
243. Korobov NV. Dalargin is an opioid-like peptide with peripheral action. *Farmakol Toksikol* 1988;51:35-38.
244. Pencheva N, Pospisek J, Hauzerova L, Barth T, Milanov P. Activity profiles of dalargin and its analogues in  $\mu$ -,  $\delta$ - and  $\kappa$ -opioid receptor selective bioassays. *Br J Pharmacol* 1999;128:569-576.
245. Maslov LN, Oeltgen PR, Lishmanov YuB, Brown SA, Barzakh EI, Krylatov AV, Pei J-M. Activation of peripheral opioid receptors increases cardiac tolerance to arrhythmogenic effect of ischemia/reperfusion. *Acad. Emer Med.* 2014;21:31-39.

246. Maslov LN, Lishmanov IuB, Naryzhnaia NV, Krylatov AV, Tam SV. Ligands of opioid and sigma receptors and correction of cardiac electrical instability in post-infarction cardiosclerosis. *Eksp Klin Farmakol* 2001;64:38-41.
247. Maslov LN, Krylatov AV, Naryzhnaia NV, Solenkova NV, Lishmanov AIu, Bogomaz SA, Gross GJ, Stefano JB, Loktiushina BA. Interactions of peripheral  $\mu$ -opioid receptors and  $K_{ATP}$ -channels in regulation of cardiac electrical stability in ischemia, reperfusion, and postinfarction cardiosclerosis. *Russ Fiziol Zh Im I M Sechenova* 2002;88:842-850.
248. Dhawan BN, Cesselin F, Raghubir R, Reisine T, Bradley PB, Portoghese PS, Hamon M. International union of pharmacology. XII. Classification of opioid receptors. *Pharmacol Rev* 1996;48:567-592.
249. Solenkova NV, Maslov LN, Budankova EV, Lishmanov AIu, Oeltgen P, Govindaswami M, Beshpalova ZhD, Ovchinnikov MV, Nagaze H. Comparative study of the antiarrhythmic activity of mu- and delta-opioid receptor agonists during acute cardiac ischemia and reperfusion models in rats. *Eksp Klin Farmakol* 2005;68:25-29.
250. Maslov LN, Lishmanov YB, Solenkova NV, Gross GJ, Stefano GB, Tam SW. Activation of peripheral delta opioid receptors eliminates cardiac electrical instability in a rat model of post-infarction cardiosclerosis via mitochondrial ATP-dependent  $K^+$  channels. *Life Sci* 2003;73:947-952.
251. Pugsley MK, Penz WP, Walker MJ, Wong TM. Antiarrhythmic effects of U-50,488H in rats subject to coronary artery occlusion. *Eur J Pharmacol* 1992;212:15-19.
252. Ugdyzhekova DS, Maslov LN, Krylatov AV, Lishmanov IuB, Tam SV. Specificity of the anti-arrhythmic effect of  $\kappa_1$ -opioid receptor agonists. *Eksp Klin Farmakol* 2001;64:17-20. (in Russian)
253. Pugsley MK, Saint DA, Penz MP, Walker MJ. Electrophysiological and antiarrhythmic actions of the kappa agonist PD 129290, and its R,R(+)-enantiomer, PD 129289. *Br J Pharmacol* 1993;110:1579-1585.
254. Pugsley MK, Saint DA, Walker MJA. An electrophysiological basis for the antiarrhythmic actions of the  $\kappa$ -opioid receptor agonist U-50,488H. *Eur J Pharmacol* 1994;261:303-309.
255. Pugsley MK, Yu EJ, Goldin AL. Potent and use-dependent block of cardiac sodium channels by U-50,488H, a benzeneacetamide kappa opioid receptor agonist. *Exp Clin. Cardiol* 2001;6:61-71.
256. Maslov LN, Lishmanov AYu, Solenkova NV, Budankova EV, Crawford D, Wong TM, Chang WC, Bray LX. The antiarrhythmic effect of (-)-U-50,488 in rats with acute ischemia and reperfusion of heart is mediated by  $\kappa_1$ -opioid receptor activation. *Eksp Klin Farmakol* 2005;68:25-29.
257. Maslov LN, Lishmanov AYu, Budankova EV, Stakheev DL, Solenkova NV, Barzakh EI, Oeltgen PR, Gross GJ, Chang W.C. Contribution of the endogenous opioid system to

- regulation of heart resistance to the arrhythmogenic effect of short-term ischemia and reperfusion. *Bull Exp Biol Med* 2005;32:375-380.
258. Mikhailova SD, Vasil'eva TV, Semushkina TM, Storozhakov GI. Role of sympathetic nervous system in protective effects of selective  $\kappa$ -opiate receptor agonist dynorphin A<sub>1-13</sub> on the incidence of cardiac arrhythmia during myocardial ischemia *Bull Exp Biol Med* 2000;129:27-29.
259. Lishmanov AY, Lasukova TV, Maslov LN, Platonov AA. The role of kappa-opioid receptors in regulation of cardiac resistance against arrhythmogenic action of ischemia and reperfusion. *Russ Fiziol Zh Im I M Sechenova* 2006;92:1419-1428.
260. Lee AYS, Zhan CY, Wong TM. Effects of  $\beta$ -endorphin on the contraction and electrical activity of the isolated perfused rat heart. *Int J Peptide Protein Res* 1984;24:525-528.
261. Lee A.Y.S. Endogenous opioid peptides and cardiac arrhythmias. *Int. J. Cardiol.* 1990; 27(2): 145-151.
262. Mikhailova SD, Storozhakov GI, Kudinova AV, Semushkina TM. Different antiarrhythmic effects of dalargin and  $\beta$ -endorphin in severe myocardial ischemia during stimulation of the sensorimotor cortex. *Bull Exp Biol Med* 1997;124:645-647.
263. Lee AYS, Wong TM. Effects of dynorphin1-13 on cardiac rhythm and cyclic adenosine monophosphate (cAMP) levels in the isolated perfused rat heart. *Neurosci Lett* 1987;80:289-292.
264. Wong TM, Lee AYS, Tai KK. Effect of drugs interacting with opioid receptors during normal perfusion or ischemia and reperfusion in the isolated rat heart - an attempt to identify cardiac opioid receptors subtype(s) involved in arrhythmogenesis. *J Mol Cell Cardiol* 1990;22:1167-1175.
265. Wu J-P, Chen Y-T, Lee AY-S Opioids in myocardial ischemia: potentiating effects of dynorphin on ischaemic arrhythmia, bradycardia and cardiogenic shock following coronary artery occlusion in the rat. *Eur. Heart J.* 1993; 14(9): 1273-1277.
266. Mikhailova SD, Glushchenko NV, Semushkina TM, Storozhakov GI. Peculiarities of ischemic cardiac arrhythmias in cats against the background of stimulation of sensorimotor cortex and administration of selective opiate receptor agonists. *Bull Exp Biol Med* 2000;129:423-424.
267. Coles JA, Sigg DC, Iaizzo PA. The role of  $\kappa$ -opioid receptor activation in pharmacological preconditioning of swine. *Am J Physiol Heart Circ Physiol* 2003;284:H2091-H2099.
268. Dumont M, Lemaire S. Characterization of non-opioid [3H]Dynorphin A-(1-13) binding sites in the rat heart. *J Mol Cell Cardiol.* 1993;25:983-991.
269. Dumont M, Lemaire S. Interactions of dynorphin A-(1-13) and nociceptin with cardiac D2 binding sites: inhibition of ischemia-evoked release of noradrenaline from synaptosomal-mitochondrial fractions. *J Mol Cell Cardiol.* 2000;32:1567-1574.

270. Nekrasova YN, Zolotarev YA, Navolotskaya EV. Detection of nonopioid  $\beta$ -endorphin receptor in the rat myocardium. *J Pept Sci* 2012;18:83-87.
271. Lishmanov YB, Maslov LN, Ugdyzhekova DS, Smagin GN. Participation of central kappa-opioid receptor in arrhythmogenesis. *Life Sci* 1997;61:PL33-PL38.
272. Lishmanov YB, Maslov LN, Ugdyzhekova DS. Participation of central and peripheral  $\kappa 1$  and  $\kappa 2$  opioid receptors in arrhythmogenesis. *Clin Exp Pharmacol Physiol* 1999;26:716-723.
273. Huang XD, Lee AYS, Wong TM. Naloxone inhibits arrhythmias induced by coronary artery occlusion and reperfusion in anaesthetized dogs. *Br J Pharmacol* 1986;87:475-477.
274. Parratt JR, Sitsapesan R. Stereospecific antiarrhythmic effect of opioid receptor antagonist in myocardial ischemia. *Br J Pharmacol* 1986;87:621-622.
275. Sitsapesan R, Parratt JR. The effects of drugs interacting with opioid receptors on the early ventricular arrhythmias arising from myocardial ischaemia. *Br J Pharmacol* 1989;97:795-800.
276. Caldwell RW, Nagarajan R, Chryssanthos A, Tuttle RR. Actions of the opioid antagonist, nalmeferene, and congeners on reperfusion cardiac arrhythmias and regional left coronary blood flow. *Pharmacology* 1990;41:161-166.
277. Murphy DB, Murphy MB. Opioid antagonist modulation of ischemia-induced ventricular arrhythmias: a peripheral mechanism. *J Cardiovasc Pharmacol* 1999;33:122-125.
278. Bolte C, Newman G, Schultz JEJ. Kappa and delta opioid receptor signaling is augmented in the failing heart. *J Mol Cell Cardiol* 2009;47(4):493-503.
279. Kasper E, Ventura C, Ziman BD, Lakatta EG, Weisman H, Capogrossi MC. Effect of U-50,488H on the contractile response of cardiomyopathic hamster ventricular myocytes. *Life Sci* 1992;50:2029-2035.
280. Yu XC, Wang HX, Wong TM. Reduced inhibitory actions of adenosine A1 and  $\kappa 1$ -opioid receptor agonists on  $\beta$ -adrenoceptors in spontaneously hypertensive rat heart. *Clin Exp Pharmacol Physiol* 1997; 24:976-977.
281. Pei JM, Wang YM, Zhu YL, Chen M, Wong TM. Signaling pathway mediated by kappa-opioid receptor is impaired in cardiac hypertrophy. *Acta Pharmacol Sin* 2001;22:887-895.
282. Hill-Pryor C, Dunbar JC. The effect of high fat-induced obesity on cardiovascular and physical activity and opioid responsiveness in conscious rats. *Clin Exp Hypertens* 2006;28:133-145.

283. Ouellette M, Brakier-Gingras L. Increase in the relative abundance of proenkephalin: a messenger RNA in the ventricles of cardiomyopathic hamsters. *Biochem Biophys Res Commun* 1988;155:449-454.
284. Dumont M, Lemaire S. Alterations of heart dynorphin-A in the development of spontaneously hypertensive rats. *Neuropeptides* 1990;15:43-48.
285. Himura Y, Liang CS, Imai N, Delehanty JM, Woolf PD, Hood WB. Short-term effects of naloxone on hemodynamics and baroreflex function in conscious dogs with pacing-induced congestive heart failure. *J Am Coll Cardiol* 1994;23:194-200.
286. Imai N, Kashiki M, Woolf PD, Liang CS. Comparison of cardiovascular effects of  $\mu$ - and  $\delta$ -opioid receptor antagonists in dogs with congestive heart failure. *Am J Physiol* 1994;267:H912-H917.
287. Forman LJ, Hock CE, Harwell M, Estilow-Isabell S. Comparison of the effects of immobilization and pressure overload induced cardiac hypertrophy on immunoreactive beta-endorphin. *Life Sci* 1995;57:2041-2047.
288. Oldroyd KG, Gray CE, Carter R, Harvey K, Borland W, Beastall G, Cobbe SM. Activation and inhibition of the endogenous opioid system in human heart failure. *Br Heart J* 1995;73:41-48.
289. Bolte C, Newman G, Schultz JEJ. Hypertensive state, independent of hypertrophy, exhibits an attenuated decrease in systolic function on cardiac  $\kappa$ -opioid receptor stimulation. *Am J Physiol Heart Circ Physiol* 2009;296:H967-H975.
290. Gross ER, Hsu AK, Gross GJ. Diabetes abolishes morphine-induced cardioprotection via multiple pathways upstream of glycogen synthase kinase-3 $\beta$ . *Diabetes* 2007;56:127-136.
291. Tsang A, Hausenloy DJ, Mocanu MM, Carr RD, Yellon DM. Preconditioning the diabetic heart. The importance of Akt phosphorylation. *Diabetes*. 2005;54:2360-2364.
292. Ludman AJ, Yellon DM, Hausenloy DJ. Cardiac preconditioning for ischaemia: lost in translation. *Dis Models Mech* 2010;3:35-38.
293. Wong TM, Lee AY. Chronic morphine treatment reduces the incidence of ventricular arrhythmias in the isolated rat heart induced by dynorphin1-13 or myocardial ischemia and reperfusion. *Neurosci Lett* 1987;77: 61-65.
294. Peart JN, Gross GJ. Chronic exposure to morphine produces a marked cardioprotective phenotype in aged mouse hearts. *Exp Gerontol* 2004;39:1021-1026.
295. Peart JN, Gross GJ. Morphine-tolerant mice exhibit a profound and persistent cardioprotective phenotype. *Circulation* 2004;109:1219-1222.
296. Peart JN, Gross GJ. Cardioprotective effects of acute and chronic opioid treatment are mediated via different signaling pathways. *Am J Physiol Heart Circ Physiol* 2006;291:H1746-H1753.



297. Skrabalova J, Neckar J, Hejnova L, Bartonova I, Kolar F, Novotny J. Antiarrhythmic effect of prolonged morphine exposure is accompanied by altered myocardial adenylyl cyclase signaling in rats. *Pharmacol Rep* 2012;64:351-359.
298. Yin W, Zhang P, Huang JH, Zhang QY, Fan R, Li J, Zhou JJ, Hu YZ, Guo HT, Zhang SM, Wang YM, Kaye AD, Gu CH, Liu JC, Cheng L, Cui Q, Yi DH, Pei JM. Stimulation of  $\kappa$ -opioid receptor reduces isoprenaline-induced cardiac hypertrophy and fibrosis. *Eur J Pharmacol* 2009; 607:135-142.
299. Robson P. Human studies of cannabinoids and medicinal cannabis. *Cannabinoids. Handbook of Experimental Pharmacology*. Ed. R. Pertwee, Springer-Verlag, 2005,168:719-756.
300. Cowan A, Zhu XZ, Mosberg HI, Omnaas JR, Porreca F. Direct dependence studies in rats with agents selective for different types of opioid receptor. *J Pharmacol Exp Ther* 1988;246:950-955.
301. Hilal-Dandan R, Brunton L. Goodman and Gilman Manual of Pharmacology and Therapeutics, 2nd ed. Section II; Neuropharmacology; Opioids, Analgesia, and Pain Management. McGraw-Hill Education. New York, Chicago, San Francisco, 2013.
302. Polonskii VM, Iarygin KN, Krivosheev OG, Moskovkin GN, Vinogradov VA. The site (central or peripheral) of the anti-ulcer action of dalargin, a synthetic analog of endogenous opioids in an experimental model of cysteamine-induced duodenal ulcer in rats. *Bull Exp Biol Med* 1987;103:433-434.
303. Mashkovsky MD. *Drugs. Manual*. Moscow: Novaya Volna; 2002.
304. Cohen MV, Downey JM. Signalling pathways and mechanisms of protection in pre- and postconditioning: historical perspective and lessons for the future. *Br J Pharmacol* 2015;172(8):1913-1932.
305. Fryer RM, Patel HH, Hsu AK, Gross GJ. Stress-activated protein kinase phosphorylation during cardioprotection in the ischemic myocardium. *Am J Physiol Heart Circ Physiol* 2001;281:H1184-H1192.
306. Murriel CL, Churchill E, Inagaki K, Szweda LI, Mochly-Rosen D. Protein kinase C $\delta$  activation induces apoptosis in response to cardiac ischemia and reperfusion damage: a mechanism involving BAD and the mitochondria. *J Biol Chem* 2004;279:47985-47991.
307. Inagaki K, Chen L, Ikeno F, Lee FH, Imahashi K, Bouley DM, Rezaee M, Yock PG, Murphy E, Mochly-Rosen D. Inhibition of  $\delta$ -protein kinase C protects against reperfusion injury of the ischemic heart in vivo. *Circulation*. 2003;108:2304-2307.
308. Liu GS, Cohen MV, Mochly-Rosen D, Downey JM. Protein kinase C- $\epsilon$  is responsible for the protection of preconditioning in rabbit cardiomyocytes. *J Mol Cell Cardiol* 1999; 31:1937-1948.
309. Soltoff SP. Rottlerin: an inappropriate and ineffective inhibitor of PKC $\delta$ . *Trends Pharmacol Sci* 2007;28:453-458.

310. Zhang Y, Chen ZW, Girwin M, Wong TM. Remifentanyl mimics cardioprotective effect of ischemic preconditioning via protein kinase C activation in open chest of rats. *Acta Pharmacol. Sin.* 2005; 26: 546-550.
311. Hausenloy DJ, Yellon DM. Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. *Heart Fail Rev* 2007;12:217-34.
312. Cao Z, Liu L, Van Winkle DM. Met5-enkephalin-induced cardioprotection occurs via transactivation of EGFR and activation of PI3K. *Am J Physiol Heart Circ Physiol* 2005;288:H1955-H1964.
313. Wu X, Zhang B, Fan R, Zhao L, Wang Y, Zhang S, Kaye AD, Huang L, Pei J. U50,488H inhibits neutrophil accumulation and TNF- $\alpha$  induction induced by ischemia-reperfusion in rat heart. *Cytokine* 2011;56:503-507.
314. Liu X, Jing G, Bai J, Yuan H. Effect of sufentanil preconditioning on myocardial P-Akt expression in rats during myocardial ischemia-reperfusion. *Nan Fang Yi Ke Da Xue Xue Bao* 2014;34:335-340.
315. Xu J, Tian W, Ma X, Guo J, Shi Q, Jin Y, Xi J, Xu Z. The molecular mechanism underlying morphine-induced Akt activation: roles of protein phosphatases and reactive oxygen species. *Cell. Biochem. Biophys.* 2011;61:303-311.
316. Fryer RM, Pratt PF, Hsu AK, Gross GJ. Differential activation of extracellular signal regulated kinase isoforms in preconditioning and opioid-induced cardioprotection. *J Pharmacol Exp Ther* 2001;296:642-649.
317. Kim JH, Jang YH, Chun KJ, Kim J, Park YH, Kim JS, Kim JM, Lee MY. Kappa-opioid receptor activation during reperfusion limits myocardial infarction via ERK1/2 activation in isolated rat hearts. *Korean J Anesthesiol* 2011;60:351-356.
318. Zhang Y, Gu EW, Zhang J, Chen ZW. Role of p38 mitogen-activated protein kinases in cardioprotection of morphine preconditioning. *Chin Med J (Engl)* 2007;120:777-781.
319. Fryer RM, Wang Y, Hsu AK, Nagase H, Gross GJ. Dependence of  $\delta$ 1-opioid receptor-induced cardioprotection on a tyrosine kinase-dependent but not a Src-dependent pathway. *J Pharmacol Exp Ther* 2001;299:477-482.
320. Peart JN, Gross GJ. Adenosine and opioid receptor-mediated cardioprotection in the rat: evidence for cross-talk between receptors. *Am J Physiol Heart Circ Physiol* 2003;285:H81-H89.
321. Peart JN, Gross GJ. Cardioprotection following adenosine kinase inhibition in rat hearts. *Basic Res Cardiol* 2005;100:328-336.
322. Headrick JP, See Hoe LE, Du Toit EF, Peart JN. Opioid receptors and cardioprotection - 'opioidergic conditioning' of the heart. *Br J Pharmacol* 2015;172:2026-2050.

323. Maslov LN, Headrick JP, Mechoulam R, Krylatov AV, Lishmanov AY, Barzakh EI, Naryzhnaya NV, Zhang Y. The role of receptor transactivation in the cardioprotective effects of preconditioning and postconditioning. *Neurosci Behav Physiol* 2013;43: 1015-1022.
324. Forster K, Kuno A, Solenkova N, Felix SB, Krieg T. The  $\delta$ -opioid receptor agonist DADLE at reperfusion protects the heart through activation of pro-survival kinases via EGF receptor transactivation. *Am J Physiol Heart Circ Physiol* 2007;293:H1604-H1608.
325. Krieg T, Cui L, Qin Q, Cohen MV, Downey JM. Mitochondrial ROS generation following acetylcholine-induced EGF receptor transactivation requires metalloproteinase cleavage of proHB-EGF. *J Mol Cell Cardiol* 2004;36:435-443.
326. Wang GY, Wu S, Pei JM, Yu XC, Wong TM.  $\kappa$ - but not  $\delta$ -opioid receptors mediate effects of ischemic preconditioning on both infarct and arrhythmia in rats. *Am J Physiol Heart Circ Physiol* 2001;280:H384-H391.
327. Cao CM, Chen M, Wong TM. The KCa channel as a trigger for the cardioprotection induced by kappa-opioid receptor stimulation - its relationship with protein kinase C. *Br J Pharmacol* 2005;145:984-991.
328. Cohen MV, Yang XM, Liu GS, Heusch G, Downey JM. Acetylcholine, bradykinin, opioids, and phenylephrine, but not adenosine, trigger preconditioning by generating free radicals and opening mitochondrial KATP channels. *Circ Res* 2001;89:273-278.
329. McPherson BC, Yao Z. Signal transduction of opioid-induced cardioprotection in ischemia-reperfusion. *Anesthesiology* 2001;94:1082-1088.
330. Obame FN, Plin-Mercier C, Assaly R, Zini R, Dubois-Rande JL, Berdeaux A, Morin D. Cardioprotective effect of morphine and a blocker of glycogen synthase kinase  $\beta$ , SB216763 [3-(2,4-dichlorophenyl)-4(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione], via inhibition of the mitochondrial permeability transition pore. *J Pharmacol Exp Ther* 2008; 326:252-258.
331. Yang XM, Liu Y, Cui L, Yang X, Liu Y, Tandon N, Kambayashi J, Downey JM, Cohen MV. Platelet P2Y<sub>12</sub> blockers confer direct postconditioning-like protection in reperfused rabbit hearts. *J Cardiovasc Pharmacol Ther* 2013;18:251-262.
332. Iliodromitis EK, Cohen MV, Dagres N, Andreadou I, Kremastinos DT, Downey JM. What is wrong with cardiac conditioning? We may be shooting at moving targets. *J Cardiovasc. Pharmacol Ther* 2015;20:357-369.